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THE EFFECTS OF IONIZING RADIATION ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

I. The Effects of Oral Administration of Mixtures of Various Sulfur-Containing Compounds on Radiation-Induced Changes in Enzyme Activities in Certain Rat Tissues

Bernard E. Hietbrink, Marjorie Keshmiri and
Mary E. Hayward

This report concerns: The results of quantitative measurements of the influence of oral administration of various combinations of cysteine, reduced glutathione (GSH), 2-aminoethylisothiouronium (AET), and 2-mercaptoethylamine (MEA) on the radiation-induced changes in enzyme activity of the spleen, thymus glands, and small intestine of the rat. The influence of sodium pentobarbital (Nembutal) on the radioprotective activity of some of these mixtures was also investigated.

Immediate or ultimate application of the results: To obtain information on the radioprotective activity of various sulfur-containing compounds when administered orally to rats. A great deal of information is available concerning the ability of this group of agents to reduce the damaging effects of radiation when given intraperitoneally or intravenously but relatively few studies have been undertaken to ascertain the protective activity of these agents given by the oral route. Information of this nature is of considerable value when considering the practical use of chemical compounds as radioprotective agents. Recent studies by Melville et al. (1) have shown that the oral administration of mixtures of cysteine and AET provides substantial protection against the lethal effects of x-irradiation and that pentobarbital (1,2,3) enhances the radioprotective activity of this mixture in rats and monkeys. Results of our recent studies (4,5) on the influence of oral administration of various radioprotective agents on the radiation-induced changes in the enzyme activities of certain hematopoietic tissues and the intestine of rats indicated that in general the compounds tested were more effective when given intraperitoneally. The current study was undertaken to obtain additional information concerning the influence of oral administration of mixtures of certain sulfur-containing compounds on the radiation-induced changes in the enzyme activities of the spleen, thymus glands, and small intestine of the rat. It is anticipated that studies of this nature may ultimately be of value in the formulation of a drug treatment that will provide optimum radioprotection when given by the oral route.

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Studies were recently undertaken in this laboratory to obtain information concerning the ability of various radioprotective compounds to reduce the radiation-induced changes in the adenosine triphosphatase activity of the spleen and thymus glands and in the cholinesterase activity of the small

intestine of the rat. Results of these studies have indicated that the oral administration of sodium diethyldithiocarbamate or dimethylammonium dimethyldithiocarbamate was not capable of reducing radiation-induced changes in the enzyme activities of these tissues when given by this route (5). The oral administration of p-aminopropiophenone (PAPP) was found to provide substantial protection to the spleen and intestine. The maximum protective effect of MEA by this route occurred at two hours after oral administration (4). Melville (3) found that the mixture of AET and cysteine, given orally, provides marked protection against the lethal effects of x-irradiation in monkeys. Thus experiments were performed to determine the ability of mixtures of various compounds to reduce the injurious effects of x-ray in the spleen, thymus glands and intestine of the rat.

The results of initial studies (6) on the influence of orally administered mixtures of cysteine and AET on the radiation-induced changes in the enzyme activities of the hematopoietic tissues and intestine of rats showed that the mixture of 1,000 mgm./kgm. of cysteine and 600 mgm./kgm. of AET provided a substantial reduction in the biological effects of 400 r in the tissues studied. Other doses of AET ranging from 300 mgm./kgm. to 700 mgm./kgm. were administered with 1,000 mgm./kgm. of cysteine but were less effective in preventing the changes in enzyme activity of one or more of the tissues studied. Preliminary experiments on the radioprotective activity of 200 mgm./kgm. or 250 mgm./kgm. of MEA given orally in a mixture with 1,000 mgm./kgm. of cysteine indicated that this mixture is considerably more effective when given intraperitoneally. The present report consists of a continuation of this study and describes the influence of oral administration of various mixtures of cysteine and MEA, reduced glutathione and MEA, AET or cysteine on the radiation-induced changes in enzyme activities of various tissues and the effect of pentobarbital on the radioprotective activity of some of these orally administered mixtures.

Materials and Methods. Adult, female Sprague-Dawley rats were used for these experiments. The animals were housed in air-conditioned quarters at 68° to 75° F. and were given Rockland Rat Diet and water ad libitum. X-irradiation was administered as a single whole body exposure with a G. E. Maximar Therapy unit employing the following radiation factors: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. giving a dose rate of 34 r to 36 r per minute as measured in air with a Victoreen ionization chamber. The compounds tested for radioprotective activity were injected as neutral aqueous mixtures. In all cases the concentrations were adjusted to permit the administration of total volumes not exceeding 1.2% of the body weight.

The adenosine triphosphatase activity of the spleens and thymus glands was measured according to the method of DuBois and Potter (7) using 0.5% homogenates of spleen and 1% homogenates of thymus glands. Assays were performed in duplicate using 0.1 ml. and 0.2 ml. of each aqueous tissue homogenate. Inorganic phosphorus was determined by the method of Fiske and Subbarow (8) and the enzyme activity was expressed as micrograms of phosphorus liberated from adenosine triphosphate by 1 mgm. of tissue during a 15-minute incubation period. The acetylcholinesterase activity of the small intestine was determined by the manometric method of DuBois and Mangun (9). A portion of the small intestine was freed from the mesenteric connective tissue and fat and longitudinally

dissected to expel the contents. The tissue was washed with distilled water, blotted with filter paper, minced and homogenized in Ringer-bicarbonate buffer. Measurements were conducted in duplicate using 50 mgm. of tissue per Warburg vessel. The vessels were gassed with 5% CO_2 and 95% N_2 for five minutes. Carbon dioxide evolution was recorded at 5-minute intervals for a period of 30 minutes following a preliminary 10-minute equilibration. Acetylcholinesterase activity was expressed as microliters of CO_2 evolved per 50 mgm. of tissue during a 10-minute incubation period. The degree of radioprotection provided by the chemical compounds in the tissues studied was expressed as per cent reduction of the biologically effective radiation dose. The data presented in this report were calculated using the dose response curves and methods described in a previous report (10).

Results

The effects of oral administration of mixtures of cysteine and 2-mercaptoethylamine on the changes in enzyme activities of the spleen, thymus glands, and intestines of rats three days after 400 r of x-irradiation. The results of preliminary studies (6) on the influence of oral administration of cysteine and MEA on the radiation-induced changes in the enzyme activities of the spleens, thymus glands, and intestines of rats indicated that mixtures of 200 mgm./kgm. or 250 mgm./kgm. of MEA with 1,000 mgm./kgm. of cysteine are more effective when given intraperitoneally. However, since no gross toxic manifestations were observed following these treatments, it was of interest to obtain information concerning the radioprotective activity of higher doses of these agents. For these experiments groups each containing four rats were given mixtures of cysteine and MEA at various intervals before 400 r of x-irradiation. Three days later the animals were sacrified for adenosine triphosphatase assays on the spleens and thymus glands and cholinesterase measurements on the intestines. The results of these measurements are presented in Table 1.

The data shown in Table 1 indicate that oral administration of mixtures of 1,000 mgm./kgm. of cysteine and 300 mgm./kgm. or 400 mgm./kgm. of MEA did not provide substantial protection to the spleen and intestine when given 30 minutes before 400 r of x-irradiation. A marked reduction in the biological effect of 400 r in the spleen was observed when 1,000 mgm./kgm. of cysteine and 500 mgm./kgm. of MEA were given at this time interval. In view of these results, studies were undertaken to obtain information on the radioprotective activity of 1,500 mgm./kgm. of cysteine given orally alone and as a mixture with 500 mgm./kgm. of MEA. Administration of 1,500 mgm./kgm. of cysteine 30 minutes before 400 r of x-ray did not significantly reduce the biological effect of 400 r in the tissues studied. In order to obtain information concerning the onset and duration of radioprotective activity of this mixture groups of animals were given 400 r of x-ray at 15 minutes, 45 minutes, and 60 minutes after receiving the chemicals. The data show that the hematopoietic tissues are not significantly protected at 15 minutes after administration of the mixture and that the radioprotective activity of these compounds is no longer evident in the tissues of animals given 400 r of x-ray 60 minutes after drug treatment.

The effects of oral administration of mixtures of reduced glutathione and other sulfur-containing compounds on the changes in enzyme activities of

TABLE 1

The Effects of Oral Administration of Mixtures of Cysteine and 2-Mercaptoethylamine on the Changes in Enzyme Activities of the Spleens, Thymus Glands, and Intestines of Rats Three Days After 400 r of X-Irradiation

Treatment	Dose in mgm./kgm.	Time of Administration Before X-ray (Minutes)	Spleen ATPase[a]		Thymus Glands ATPase[a]		Intestinal Cholinesterase[b]	
			400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction
None	.....	..	51.4±3.0	..	20.2±1.1	..	69± 9	..
Cysteine plus MEA	1,000 300	30	49.8±1.4	9	17.7±0.4	19	70± 5	0
Cysteine plus MEA	1,000 400	30	49.9±2.2	9	20.8±1.4	0	70± 2	0
Cysteine plus MEA	1,000 500	30	43.1±1.4	37	19.2±2.5	9	66± 3	0
Cysteine	1,500	30	49.2±1.3	12	19.7±1.8	4	75± 7	10
Cysteine plus MEA	1,500 500	30	45.2±2.3	31	17.6±1.5	19	97±13	36
Cysteine plus MEA	1,500 500	15	49.4±1.4	11	21.1±0.7	0	85± 7	23
Cysteine plus MEA	1,500 500	45	46.2±1.8	26	19.9±1.6	3	71± 3	0
Cysteine plus MEA	1,500 500	60	53.2±3.2	0	23.2±0.8	0	75± 7	10

[a] Activity expressed as µgm. of P liberated from ATP/mgm. of tissue/15 minutes.

[b] Activity expressed as µl. of CO_2 evolved/50 mgm. tissue/10 minutes.

the spleens, thymus glands, and intestines of rats three days after 400 r of x-irradiation. Results of experiments presented above and in our previous report (6) illustrate that the oral administration of mixtures of cysteine and MEA and cysteine and AET at appropriate intervals before x-irradiation provides substantial reductions in the biological effects of radiation in the spleens, thymus glands, and intestines of rats. Therefore, studies were undertaken to obtain information on the radioprotective activity resulting from oral administration of mixtures of glutathione and other sulfur-containing agents. For these experiments groups each containing four rats were given mixtures of glutathione and MEA, glutathione and AET, or glutathione and cysteine at various intervals prior to 400 r of x-irradiation. Three days later the animals were sacrificed and the enzyme activities of the spleens, thymus glands, and intestines were determined. The results of these determinations are shown in Table 2.

The data presented in Table 2 show that the oral administration of 1,500 mgm./kgm. of reduced glutathione provided 19% and 12% reductions in the biological effect of 400 r in the spleens and thymus glands respectively when given 30 minutes before x-irradiation. Substantial protection was afforded to the tissues studied when the mixture of 1,500 mgm./kgm. of glutathione and 500 mgm./kgm. of MEA was given 15 minutes prior to radiation. Results of experiments undertaken to obtain information concerning the duration of radioprotective activity indicate that this mixture provides significant protection to the spleens and thymus glands but did not reduce the injurious effects of x-ray on the intestines of rats irradiated at 30 and 60 minutes after oral administration of the mixture. Mixtures of glutathione plus AET and glutathione plus cysteine did not significantly alter the radiation-induced changes in the spleens and intestines caused by 400 r but provided 18% and 20% reductions respectively in the biological effect of x-irradiation in the thymus glands.

The influence of sodium pentobarbital and mixtures of various sulfur-containing compounds on the changes in enzyme activities of the spleens, thymus glands, and intestines of rats three days after 400 r of x-irradiation. Melville et al. (1,2) have recently found that intraperitoneal or intravenous injections of pentobarbital enhanced the radioprotective activity of orally administered AET or AET and cysteine mixtures as measured by survival studies in rats and monkeys. Results of previous studies (6) in this laboratory showed that the mixture of 1,000 mgm./kgm. of cysteine and 600 mgm./kgm. of AET given orally provides substantial reductions in the biological effects of 400 r in the tissues studied. To obtain further information concerning the influence of pentobarbital on the radioprotective activity of mixtures of AET and cysteine in specific tissues, groups of four rats were given various mixtures of these compounds orally 30 minutes before 400 r of x-ray and 25 mgm./kgm. of pentobarbital was given intraperitoneally 20 minutes later. Enzyme assays were performed on the tissues of these animals three days later. The results of these and other experiments are shown in Table 3.

The data presented in Table 3 show that the oral administration of 1,000 mgm./kgm. of cysteine provided a 26% reduction in the biological effect of 400 r of x-ray in the spleen but did not significantly benefit the thymus glands and intestines and that 25 mgm./kgm. of pentobarbital abolished the

TABLE 2

The Effects of Oral Administration of Mixtures of Reduced Glutathione and Other Sulfur-Containing Compounds on the Changes in Enzyme Activities of the Spleens, Thymus Glands and Intestines of Rats Three Days After 400 r of X-Irradiation

Treatment	Dose in mgm./kgm.	Time of Administration Before X-ray (Minutes)	Spleen ATPase[a]		Thymus Glands ATPase[a]		Intestinal Cholinesterase[b]	
			400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction
None	.....	..	51.4±3.0	..	20.2±1.1	..	69±9	..
Glutathione	1,500	30	47.2±2.6	19	18.8±0.8	12	63±4	0
Glutathione plus MEA	1,500 500	15	44.1±1.2	35	17.6±0.6	20	92±9	29
Glutathione plus MEA	1,500 500	30	47.3±3.6	19	18.5±1.5	14	70±3	0
Glutathione plus MEA	1,500 500	45	45.5±0.6	29	17.4±0.7	21	70±7	0
Glutathione plus MEA	1,500 500	60	46.7±2.6	24	18.5±0.9	14	75±7	10
Glutathione plus AET	1,500 600	30	49.6±4.7	10	17.8±1.5	18	75±4	10
Glutathione plus cysteine	1,500 1,000	30	50.7±0.3	0	17.0±1.9	22	66±4	0

[a] Activity expressed as µgm. of P liberated from ATP/mgm. of tissue/15 minutes.

[b] Activity expressed as µl. of CO_2 evolved/50 mgm. tissue/10 minutes.

TABLE 3

The Influence of Sodium Pentobarbital and Mixtures of Various Sulfur-Containing Compounds on the Changes in Enzyme Activities of the Spleens, Thymus Glands and Intestines of Rats Three Days After 400 r of X-Irradiation

Treatment	Dose in mgm./kgm.	Time of Administration Before X-ray (Minutes)	Spleen ATPase[a]		Thymus Glands ATPase[a]		Intestinal Cholinesterase[b]	
			400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction
None	.....	..	51.4±3.0	..	20.2±1.1	..	69± 9	..
Cysteine	1,000	30	46.2±2.5	26	20.9±0.9	0	74± 5	6
Cysteine plus pentobarbital	1,000 25	30 10[c]	52.2±2.5	0	20.7±0.4	0	69± 3	0
Cysteine plus AET plus pentobarbital	1,000 600 25	30 10	46.0±1.0	27	19.1±0.9	9	70± 8	0
Cysteine plus AET plus pentobarbital	500 600 25	30 10	46.5±2.3	25	19.7±0.2	4	63± 8	0
Cysteine plus AET plus pentobarbital	1,000 400 25	30 10	43.2±2.1	37	18.0±0.1	16	86±14	23
Cysteine plus AET plus pentobarbital	500 400 25	30 10	46.2±4.7	26	17.4±0.9	20	69± 2	0
Cysteine plus MEA plus pentobarbital	1,000 500 25	30 10	42.9±3.1	39	19.7±1.5	4	61± 7	0

[a] Activity expressed as μgm. of P liberated from ATP/mgm. of tissue/15 minutes.

[b] Activity expressed as μl. of CO_2 evolved/50 mgm. tissue/10 minutes.

[c] In all instances sodium pentobarbital was administered by the intraperitoneal route.

protective activity of cysteine in the spleens. Data presented in our previous report (6) showed that the mixture of 1,000 mgm./kgm. of cysteine and 600 mgm./kgm. of AET provided marked dose reductions in all the tissues studied. Intraperitoneal injection of pentobarbital reduced the beneficial effects of the mixture in the hematopoietic tissues and totally inhibited the protection observed in the intestine. Melville and Leffingwell (2) have shown that the combination of AET, pentobarbital, and x-irradiation caused additive or even synergistic lethal effects. To determine whether manifestations of drug toxicity reduced the radioprotective activity of this mixture, groups of animals were given various dosage levels of AET and cysteine. It was found that reducing the level of AET in the mixture to 400 mgm./kgm. enhanced the protective activity of this treatment in all the tissues studied. However, when cysteine or both cysteine and AET were reduced this mixture did not alter the effect of 400 r in the intestine. Administration of pentobarbital to animals previously treated with the mixture of 1,000 mgm./kgm. of cysteine and 500 mgm./kgm. of MEA did not significantly affect the protective activity of this mixture (Table 1).

Discussion

This investigation consisted of a continuation of experiments recently undertaken to determine the effect of oral administration of various mixtures of cysteine, AET, MEA, or glutathione on the radiation-induced changes in the adenosine triphosphatase activity of the spleens and thymus glands and in the cholinesterase activity of the small intestine of rats. The results of these experiments showed that the mixture of 1,500 mgm./kgm. of cysteine and 500 mgm./kgm. of MEA given 30 minutes before x-irradiation provided substantial reduction in the biological effects of 400 r in the tissues studied. Treatment of the animals at other time intervals before radiation exposure with other dosage levels of this mixture failed to be as effective. The mixture of 1,500 mgm./kgm. of glutathione and 500 mgm./kgm. of MEA given 15 minutes prior to x-ray was the most effective dosage schedule of this mixture tested in reducing the biological effects of radiation in the spleen and intestine. Administration of this mixture at 30 minutes to 60 minutes before radiation provided substantial protection to the hematopoietic tissues but did not reduce the damaging effect of 400 r in the intestines.

Studies were also undertaken to obtain information concerning the influence of sodium pentobarbital on the ability of mixtures of cysteine and AET to reduce the biological effect of radiation in the hematopoietic tissues and intestines of the rat. These experiments were prompted by the recent findings of Melville and Leffingwell (2) who showed that intraperitoneal injections of pentobarbital enhance the protective activity of orally administered AET against the lethal effects of ionizing radiations in rats. These investigators observed that the degree of radioprotective activity obtained was dependent upon adjustment of the dosage of the sulfur-containing compounds and the sedative in order to eliminate the toxic manifestations of the protective treatment. The results of studies presented in the current report illustrate these observations. The protective activity provided the intestine by the mixture of 1,000 mgm./kgm. of cysteine and 600 mgm./kgm. of AET was not observed when pentobarbital was administered ten minutes before radiation but significant protection was obtained in the tissues studied when the dose of AET was reduced to 400 mgm./kgm.

The results of these studies illustrate that various mixtures of sulfur-containing compounds are capable of reducing the damaging effects of x-irradiation in the hematopoietic tissues and intestine but in most instances it is more difficult to obtain protective activity by oral administration than it is when the agents are given intraperitoneally. Thirty-day survival studies, which will assist in the evaluation of the effectiveness of these orally administered mixtures, are currently in progress.

Summary

1. A study was conducted to quantitatively determine the radioprotective activity of orally administered mixtures of various sulfur-containing compounds in rats. Doses of MEA ranging from 300 mgm./kgm. to 500 mgm./kgm. were given as mixtures with 1,000 mgm./kgm. of cysteine 30 minutes before 400 r of x-irradiation. The mixture of 1,000 mgm./kgm. of cysteine and 500 mgm./kgm. of MEA provided a reduction of 37% in the biological effect of radiation in the spleens but like the other mixtures tested did not substantially benefit the thymus glands and intestines. Oral administration of 1,500 mgm./kgm. of cysteine and 500 mgm./kgm. of MEA 30 minutes before x-ray provided marked reductions in the biological effect of 400 r in the tissues tested. This mixture was much less effective when given 15 minutes, 45 minutes, or 60 minutes before x-ray.

2. Experiments undertaken to determine the protective activity of mixtures of glutathione and other sulfur-containing compounds indicated that the mixture of 1,500 mgm./kgm. of glutathione and 500 mgm./kgm. of MEA given orally 15 minutes before x-ray provided 35%, 20%, and 29% reductions in the biological effect of 400 r in the spleens, thymus glands, and intestines respectively. When this treatment was given at 30 minutes, 45 minutes, or 60 minutes before x-ray, it provided protection to the hematopoietic tissues but did not reduce the injurious effects in the intestines. Mixtures of glutathione plus AET and glutathione plus cysteine did not significantly alter the radiation-induced changes in the ensyme activities of the spleen and intestine when given orally 30 minutes before 400 r.

3. Results of studies to determine the influence of intraperitoneal injection of sodium pentobarbital on the radioprotective activity of chemical agents given orally showed that 25 mgm./kgm. of pentobarbital nullified the protective effect of 1,000 mgm./kgm. of cysteine in the spleen. In most instances pentobarbital did not significantly affect the protective activity of the mixtures in the hematopoietic tissues but reduced the beneficial effects of these agents in the intestine.

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THE EFFECTS OF IONIZING RADIATION ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

II. Influence of X-irradiation on the Development of a Thiophosphate-Oxidizing Enzyme System in the Livers of Young Male Rats

Bernard E. Hietbrink, Marjorie Keshmiri and Kenneth P. DuBois

This report concerns: Results of experiments undertaken to obtain additional information concerning the influence of ionizing radiations on the development of the phosphorothioate-oxidizing enzyme in the livers of young male rats. Observations were made on the influence of shielding the head and testes on the radiation-induced inhibition of the development of the drug metabolizing enzyme. The influence of sodium pentobarbital and sodium phenobarbital on the development of this enzyme system and the effect of x-irradiation on phenobarbital-induced enzyme synthesis were also investigated.

Immediate or ultimate application of the results: The present investigation constitutes a continuation of studies recently initiated to obtain information on the effects of ionizing radiations on the development of microsomal enzymes in the liver which are responsible for the oxidative desulfuration of phosphorothioates and which catalyze the metabolism of other drugs and toxic compounds. Previously we have attempted to obtain information concerning the influence of x-irradiation and gamma radiation on the mechanisms responsible for the normal development of these microsome enzymes in young male rats. Studies in this (1) and other laboratories (2,3,4) have shown that polycyclic hydrocarbons and other foreign chemical agents enhance the activity of liver microsome enzymes which are responsible for the metabolism of various drugs in the intact animal. Thus a considerable portion of the present report is concerned with the results of studies on the effect of x-irradiation on drug-induced stimulation of the activity of drug metabolizing enzymes. Results of initial studies on the influence of radiation on drug-induced stimulation of enzyme activity indicate that 200 r or 400 r of x-ray given 24 hours before initiating a series of daily injections of various doses of sodium phenobarbital does not substantially affect the degree of phenobarbital-induced stimulation in the activity of the phosphorothioate-oxidizing enzymes in the livers of adult female and young male rats. Evidence is presented that shielding the head and testes while the remainder of the body is exposed to radiation reduces the degree of inhibition in the development of enzyme activity caused by 200 r of x-ray. It is anticipated that information obtained from these experiments will ultimately assist in ascertaining the biological mechanisms involved in the injurious action of ionizing radiations on mammalian tissues.

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A systematic study was recently undertaken (5) to obtain information concerning the radiation-induced defect in the development of microsome enzymes which are responsible for the metabolism of various drugs and toxic agents in

the livers of young male rats. Results have indicated that doses of x-ray as low as 100 r (6,7) cause substantial reductions in the rate of development of the enzyme system, that the inhibition is reversible, and that the enzyme activity reaches normal adult levels at five to six weeks after 100 r or 200 r of x-irradiation (8). Administration of a second dose of 100 r or 200 r of x-irradiation to 30-day old rats at seven days after an initial exposure increased the period required for synthesis of enzyme activity to normal adult levels; however, when the second dose was given 14 days after the first exposure the microsome oxidases developed at a rate similar to that of animals irradiated only at 23 days of age.

Shielding various areas during x-ray exposure was used in an attempt to gain information concerning the gross site of radiation-induced inhibition. These experiments showed that shielding the testes or the liver area did not prevent the inhibitory effect of 200 r or 400 r of x-ray on the development of the drug metabolizing enzyme (7). It was also found that the administration of 600 r (7) or 800 r (9) of x-ray to the liver area while shielding the remainder of the body substantially reduced the rate of development of the enzyme system during the latter part of the observation period. The present report describes results of experiments on the influence of shielding the head and testes on the inhibitory effect of 200 r of x-ray on the synthesis of this enzyme system.

In our previous studies we have been interested in obtaining information on the influence of ionizing radiations on the normal rate of development of microsomal enzyme systems. Recent studies by Conney et al. (2) show that pretreatment of young rats with drugs differing widely in chemical structure and pharmacological activity stimulates the activity of drug-metabolizing enzymes in microsomes of the liver. Measurements were made of the effect of x-irradiation on the barbiturate-induced stimulation of the synthesis of the phosphorothioate-oxidizing enzyme system in the liver of young male and adult female rats. Results of these measurements indicated that administration of x-ray one day before daily injection of phenobarbital does not significantly affect the drug-induced stimulation in enzyme activity.

Materials and Methods. Adult female and young male Sprague-Dawley rats were used for these experiments. The animals were housed in air-conditioned quarters and were given Rockland Rat Diet and water ad libitum. X-irradiation was administered as a single exposure with a G. E. Maximar therapy unit employing the following radiation factors: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. giving a dose rate of 34 r to 36 r per minute as measured in air with a Victoreen ionization chamber. For experiments on the effect of partial body shielding on the development of the drug metabolizing enzyme system, weanling rats (23 days old) were anesthetized with aqueous solutions of sodium pentobarbital (25 mgm./kgm. intraperitoneally) to facilitate accurate placement and maintenance of the lead shields during radiation exposure. Aqueous solutions of sodium phenobarbital were given intraperitoneally.

For enzyme assays the rats were sacrificed by decapitation and the livers were quickly removed, weighed, and homogenized in cold distilled water. Guthion was converted to its active metabolite by the method developed by Murphy and DuBois (10) in this laboratory and by a modification of the method

used by Conney et al. (4) for other reactions catalyzed by microsome enzymes. The details of these modifications and the methods employed in the calculation of the enzyme activity have been described in detail in a previous report from this laboratory (11).

Results

The influence of partial body shielding on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. In recent studies we employed partial body shielding to obtain information on the gross site of action of radiation in connection with the inhibition of the development of microsome oxidases. It was found that 200 r or 400 r of x-irradiation given to the liver area only does not inhibit development of the enzyme system in contrast to the marked inhibition resulting from these doses of whole body radiation. Testosterone stimulates the development of the enzyme system (10) but shielding the testes during irradiation did not prevent the radiation-induced inhibition of development of the enzyme system. Evidence was obtained which indicates that radiation injury to the adrenal glands may be a factor in the delayed development of enzyme activity. In this connection daily injections of adrenal cortex extract reduced the degree of radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme caused by 200 r of total-body x-ray. Experiments were undertaken to obtain additional information on the effect of partial-body shielding on the development of microsomal enzyme activity in young male rats. For these experiments 23-day old male rats were anesthetized with 25 mgm./kgm. of sodium pentobarbital and lead shields were placed so as to shield the head and the testes. The remainder of the body was then given 200 r of x-irradiation. The animals were sacrificed at various intervals during the following three weeks, a portion of the liver was removed and the microsome oxidase activity was measured. The results of these measurements are presented in Figure 1 where each point on the curves is the average of measurements on the livers of at least four animals.

The data in Figure 1 show that shielding the head and testes reduces the degree of inhibition caused by 200 r of x-ray. Since previous studies have shown that shielding the testes does not alter the inhibitory action of 200 r, these results provide evidence that radiation-induced injury to the head may be partially responsible for the inhibitory action of x-ray in the liver. Additional experiments, however, must be undertaken to more accurately determine the influence of shielding and irradiating the head area on the development of drug metabolizing enzymes.

The influence of 25 mgm./kgm. of sodium pentobarbital on the development of the phosphorothioate-oxidizing enzymes in the livers of young male rats. It was noted in this and previous studies (7,8,9) that the activity of the microsome enzymes of the livers of partially shielded irradiated rats was substantially elevated for approximately 10 to 12 days following x-ray. It was suggested (9) that sodium pentobarbital used to anesthetize the animals during the shielding experiments may be causing this initial stimulation in activity since Conney et al. (2) have shown that relatively small doses of other barbiturates cause marked increases in the activity of various drug-metabolizing enzymes which are located in the microsomes of the liver. To determine the effect of

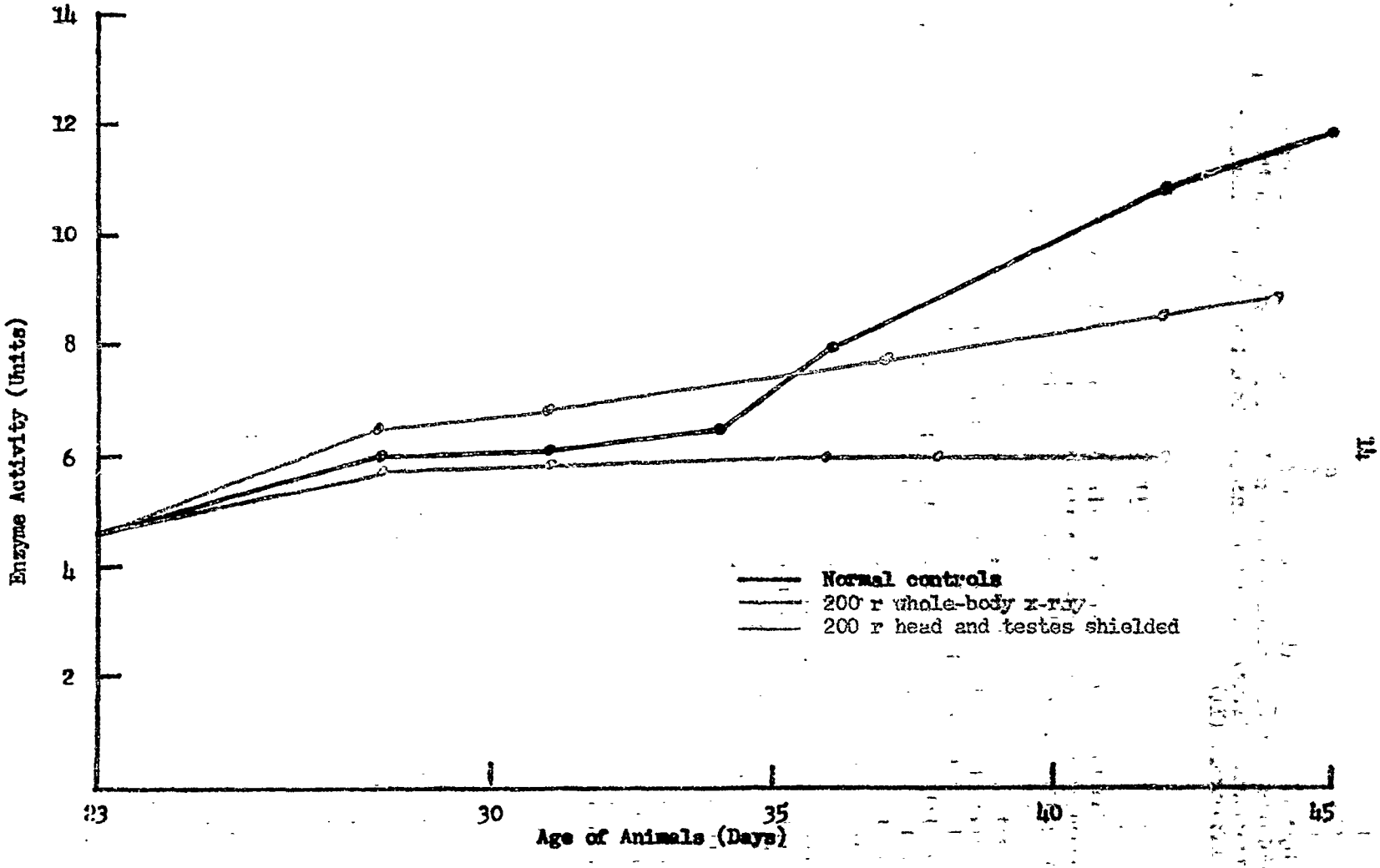

Figure 1. The influence of partial body shielding on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats.

barbiturates on the development of the phosphorothioate-oxidizing enzyme in the livers of young male rats a group of 23-day old animals was given 25 mgm./kgm. of sodium pentobarbital ten minutes before 200 r of x-irradiation. Another group consisting of non-irradiated animals was given 25 mgm./kgm. of pentobarbital. The animals were sacrificed at various intervals during the following three weeks and the microsome oxidase activity of the livers was measured. The results of these measurements are shown in Figure 2 where each point on the curves is the average of measurements on the livers of at least four animals.

The data presented in Figure 2 illustrate the marked stimulation in enzyme activity observed at one day after injection of pentobarbital in both the irradiated and non-irradiated animals. The activity tended to return toward normal after five to six days and no significant difference was found in the enzyme activity of the pentobarbital treated and non-treated animals given 200 r of x-ray during the last two weeks of the observation period. The results of this study indicate that the early stimulation in enzyme activity observed in animals anesthetized for shielding studies was due to pentobarbital-induced enzyme synthesis.

The influence of x-irradiation on phenobarbital-induced stimulation of the phosphorothioate-oxidizing enzyme in the liver of young male rats. Conney et al. (2) have recently found that relatively small doses of phenobarbital cause a marked increase in the activity of the enzymes responsible for the demethylation of 3-methyl-4-monomethylaminoazobenzene (3-methyl-MAB) and for the metabolism of zoxazolamine and other chemicals in the livers of young male rats. Thus a study was undertaken to obtain information concerning the influence of x-irradiation on the barbiturate-induced stimulation in microsome oxidase activity. The results of studies presented above indicated that pentobarbital causes a marked stimulation in the activity of the microsome oxidase which catalyzes the metabolism of phosphorothioates. Preliminary studies showed that daily 2 mgm./kgm. doses of phenobarbital cause marked stimulation in the development of enzyme activity. Groups of 23-day old male rats were given 200 r of x-ray and 24 hours later daily injections of phenobarbital (2 mgm./kgm.) were begun. Normal and drug-treated unirradiated animals served as the controls. Animals were sacrificed for enzyme measurements at 28, 30, and 32 days of age. The results of these measurements are shown in Figure 3 where each bar represents the average and range obtained from the number of animals indicated in parenthesis.

The data in Figure 3 indicate that phenobarbital causes approximately a two-fold increase in the normal rate of development of the phosphorothioate-oxidizing enzyme system after 4, 6, and 8 daily 2 mgm./kgm. injections. The phenobarbital-induced stimulation in enzyme activity was less marked in animals that had received 200 r at 23 days of age; however, the difference was not significant due to the variability of the responses.

In view of these results it was of interest to determine the influence of increased levels of phenobarbital and higher doses of x-irradiation on the development of the drug-metabolizing enzymes. For these experiments groups of 23-day old rats were given 200 r or 400 r of x-irradiation and daily injections of 5 mgm./kgm. of phenobarbital were begun 24 hours later. The animals were

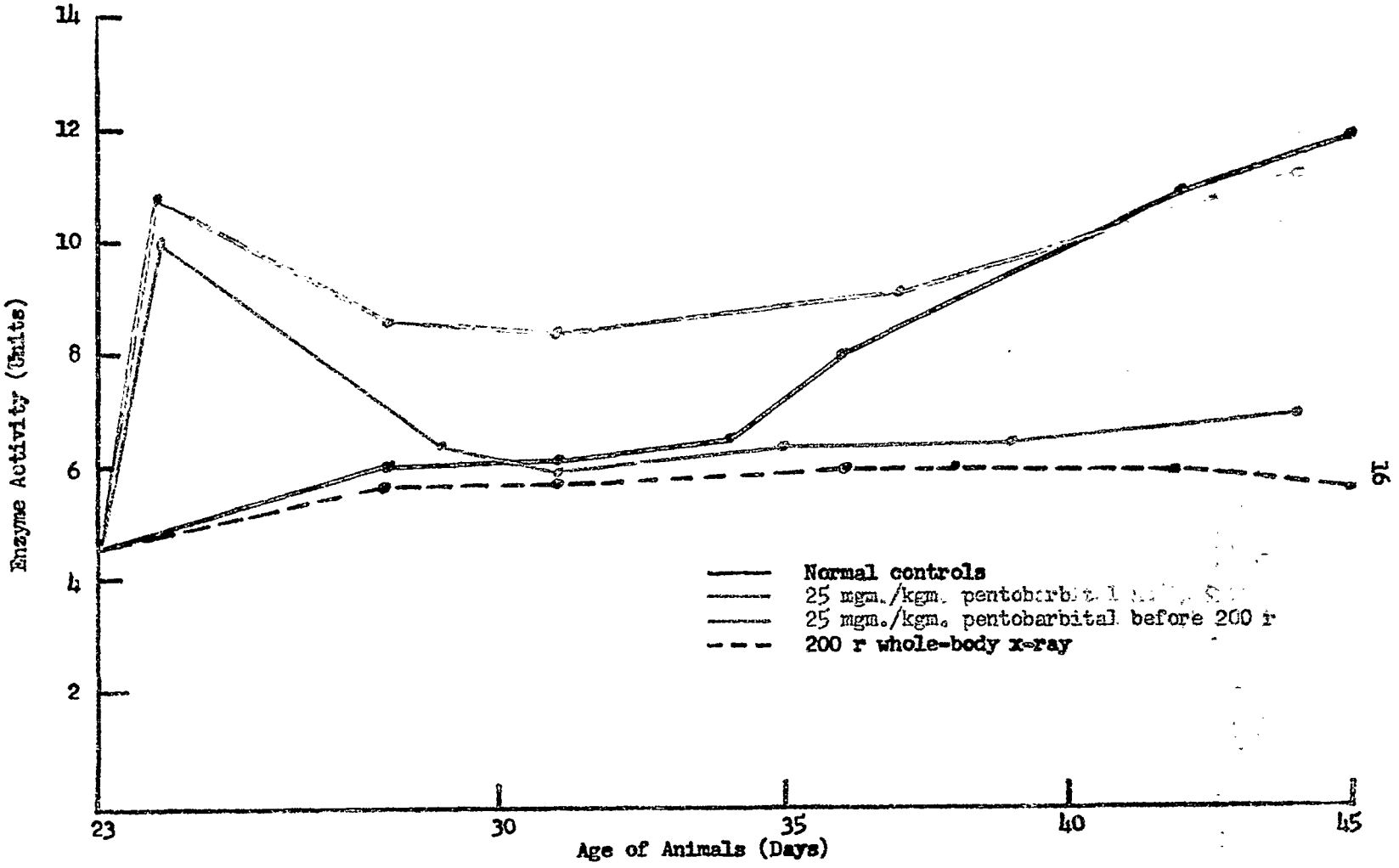

Figure 2. The influence of 25 mgm./kgm. of sodium pentobarbital on the development of the phosphorothioate-oxidizing enzymes in the livers of young male rats.

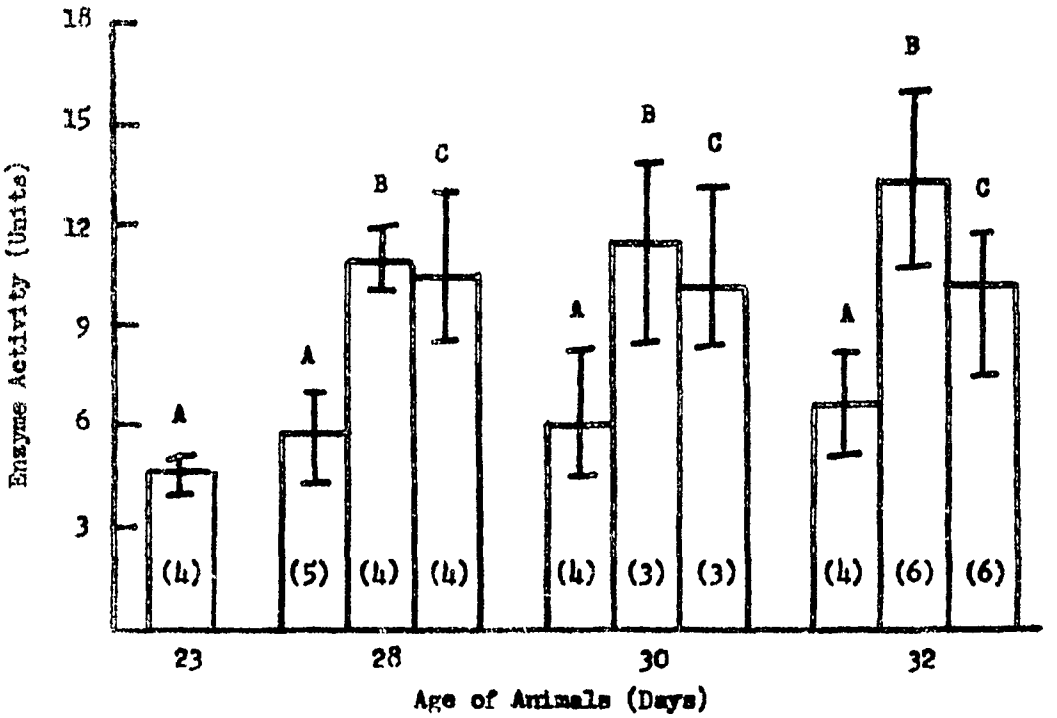

Figure 3. The influence of 200 r of x-irradiation on phenobarbital-induced stimulation of the phosphorothioate-oxidising enzyme in the livers of young male rats. A, control activity; B, 2 mgm./kgm. of phenobarbital per day starting at 24 days of age; C, 200 r at 23 days of age, 2 mgm./kgm. of phenobarbital per day starting at 24 days of age. Numbers in parenthesis represent the number of animals in each group.

sacrificed 4, 6, and 8 days later and the drug-metabolizing enzyme activity of the livers was measured. The results of these measurements are presented in Figure 4 where each bar represents the average and range obtained for the livers of each group of animals. The number of animals in each group is given in parenthesis.

The data presented in Figure 4 indicate that 5 mgm./kgm. of phenobarbital cause a two- to three-fold increase in the synthesis of enzyme activity after 4, 6, and 8 daily doses of 5 mgm./kgm. of phenobarbital. It is apparent that 200 r of x-irradiation does not inhibit the drug-induced synthesis of enzyme activity; however, the enzyme activity of the animals given 400 r of x-ray at 23 days and 6 or 8 daily injections of phenobarbital was substantially less than the unirradiated drug-treated controls.

The influence of x-irradiation on phenobarbital-induced stimulation of the phosphorothioate-oxidizing enzyme in the liver of the rat. Experiments were undertaken to obtain information concerning the effect of daily injections of phenobarbital and x-irradiation on the synthesis of phosphorothioate-oxidizing enzymes in the livers of adult female rats. For these studies groups of female rats were given 600 r of x-ray and injections of 37.5 mgm./kgm. of phenobarbital were given twice daily (75 mgm./kgm./day) starting 24 hours later. Young male rats (23 days old) were given 400 r and placed on the same injection schedule. Four days later the animals were sacrificed and the drug metabolizing activity of the livers was measured. The results of these measurements are shown in Figure 5. Each bar represents the average of 4 to 8 animals as indicated in parenthesis and the range of the results.

The results of experiments presented in Figure 5 show that daily injections of 75 mgm./kgm. of phenobarbital caused approximately a two-fold increase in the activity of the liver of adult females and a four-fold increase in the enzyme activity of young male rats. The livers of female rats given 600 r and male rats given 400 r of x-ray before daily injections of 75 mgm./kgm. of phenobarbital exhibited a similar degree of enzyme induction to that observed in the drug-treated, unirradiated animals. Thus the results of these studies indicate that administration of phenobarbital causes marked increases in the synthesis of the phosphorothioate-oxidizing enzyme system in adult female and young male rats, that this increase is dose dependent in young male rats (Figures 3, 4, and 5) and that sublethal doses of x-irradiation do not significantly influence the degree of phenobarbital-induced increases in activity.

Discussion

The present investigation consisted of experiments undertaken to obtain additional information on the influence of ionizing radiations on the normal development and the drug-induced stimulation of an enzyme responsible for the oxidative metabolism of certain chemical agents by the livers of adult female and young male rats. The present report describes further studies on the effect of partial body shielding on the inhibitory effect of x-ray on the development of microsome oxidases, the influence of sodium pentobarbital, used as an anesthetic agent, on the development of enzyme activity and the effect of

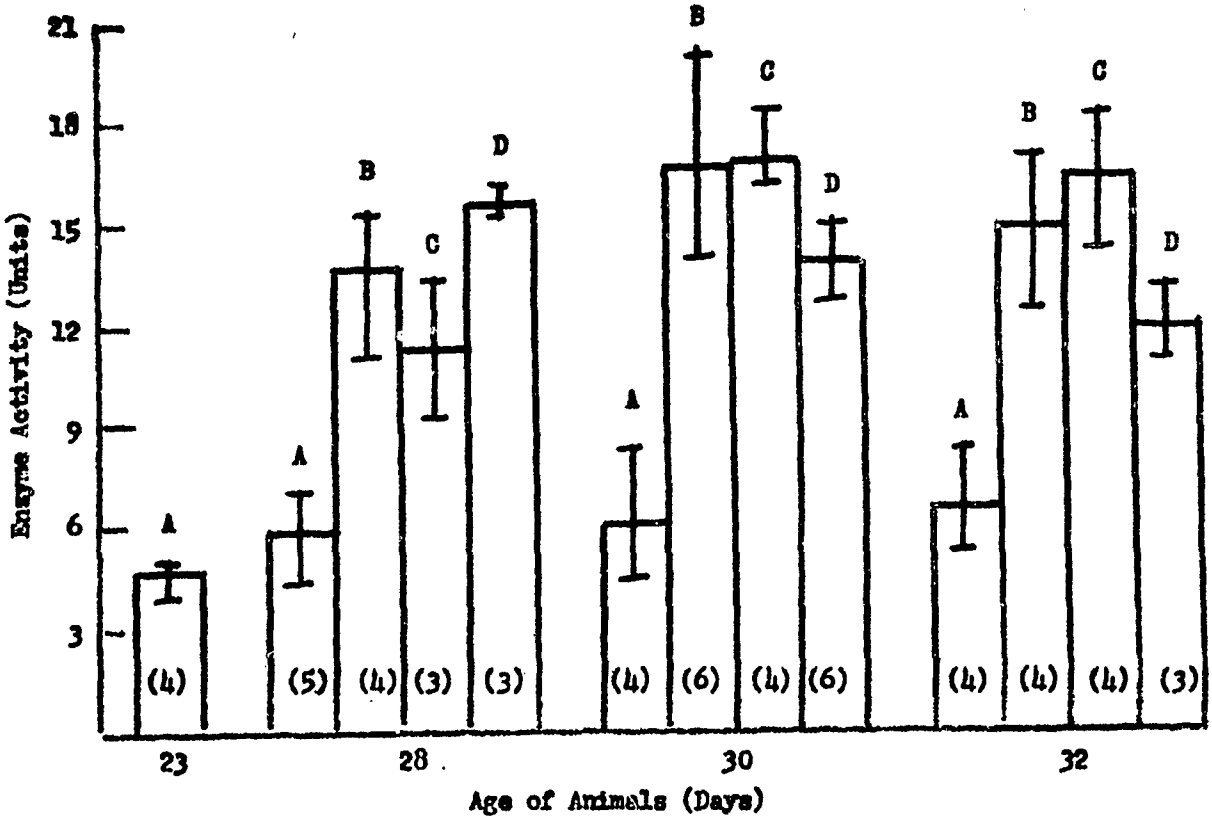

Figure 4. The influence of x-irradiation on phenobarbital-induced stimulation of the phosphorothioate-oxidizing enzyme in the livers of young male rats. A, control activity; B, 5 mgm./kgm. of phenobarbital per day starting at 24 days of age; C, 200 r at 23 days of age and 5 mgm./kgm. of phenobarbital per day starting at 24 days of age; D, 400 r at 23 days of age and 5 mgm./kgm. of phenobarbital per day starting at 24 days of age. The number in parenthesis represent the number of animals in each group.

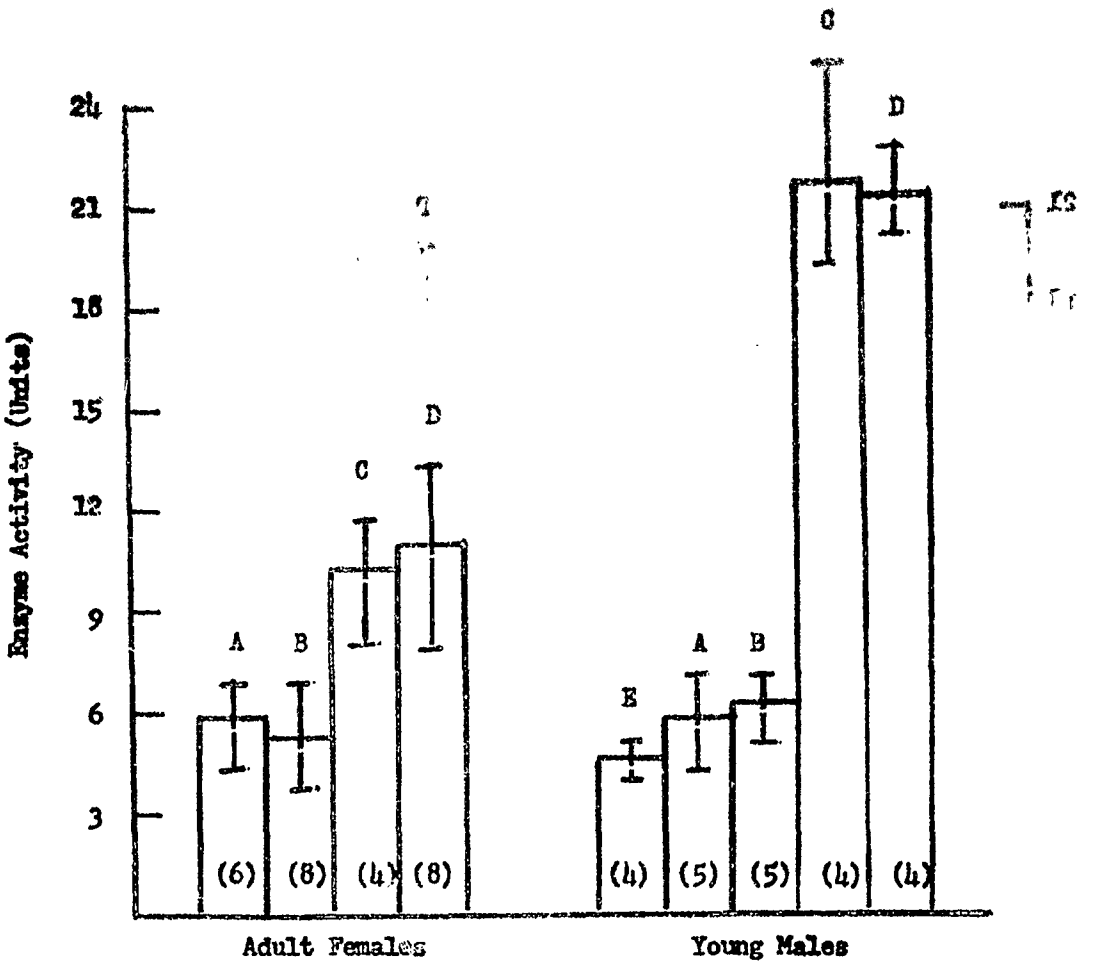

Figure 5. The influence of x-irradiation on phenobarbital-induced stimulation of the phosphorothioate-oxidising enzyme in the livers of rats. A, control activity; B, x-irradiated animals, adult females (600 r), young males (400 r); C, 75 mgm./kgm. of phenobarbital per day for 4 days; D, adult females (600 r), young males (400 r) plus 75 mgm./kgm. of phenobarbital per day for 4 days, E, 23-day old control activity. The numbers in parenthesis represent the number of animals in each group.

ionizing radiations on the sodium phenobarbital-induced stimulation in development of these enzymes. The results of studies on the influence of partial body shielding on the development of enzyme activity indicated that shielding the head and testes while exposing the remainder of the body to x-irradiation substantially reduced the inhibitory effect of 200 r on the development of the phosphorothioate-oxidizing enzyme in the livers of total-body and testes shielded irradiated animals. The mechanisms responsible for the reduction in the biological effectiveness of radiation caused by shielding the head area are not readily apparent. However, it is anticipated that studies currently in progress will supply information which will aid in ascertaining the influence of radiation to the head area on the development of microsome enzyme activity in the livers of young male rats.

It was noted in recent studies (7,8,9,11) that radiation appeared to enhance the development of the phosphorothioate-oxidizing enzymes in the livers of partially shielded animals for approximately 10 to 12 days following x-ray. The results of studies by Conney et al. (2), who found that small doses of some barbiturates cause marked increases in the activity of various drug-metabolizing enzymes located in the microsome fraction of the liver, suggested that the pentobarbital used to anesthetize these animals during irradiation caused the initial stimulation in activity. Results of experiments on the influence of pentobarbital on enzyme synthesis indicated that the early stimulation in drug metabolizing enzyme activity was caused by the anesthetic agent.

The absence of radiation-induced inhibition of the stimulatory effect of phenobarbital is somewhat surprising. Radiation has been found to inhibit the growth rate or decrease the size of various tissues (i.e., spleen, thymus glands, testes, and other rapidly proliferating tissues). Phenobarbital-induced stimulation in enzyme activity of the liver has been shown to coincide with marked increases in liver weight and elevated microsomal and total liver protein (2). Further studies are currently in progress to determine whether the time interval between radiation and initiation of barbiturate injections has any influence on the synthesis of enzyme activity.

Summary

1. Additional studies were undertaken to determine the radiosensitivity of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. The results of experiments on the influence of partial body shielding indicated that shielding the head and testes reduced the degree of inhibition caused by 200 r of x-ray.

2. The injection of 25 mgm./kgm. of sodium pentobarbital caused a marked stimulation in the microsome enzyme activity of the livers of normal and irradiated young male rats at 24 hours after administration. The enzyme activity tended to return toward normal in both groups after five to seven days. It is evident that the increase in enzyme activity observed in irradiated animals, which had been anesthetized with pentobarbital during x-ray exposure, was due to barbiturate-induced enzyme stimulation.

3. Measurements of the influence of x-irradiation on phenobarbital-induced increases in phosphorothioate-oxidase activity showed that 200 r does not significantly affect the increase in enzyme activity caused by daily doses of 2 mgm./kgm. of the barbiturate. Results of experiments on the influence of 200 r or 400 r of x-irradiation on the increase in enzyme activity caused by 5 mgm./kgm. of phenobarbital indicated that 200 r did not influence barbiturate-induced stimulation in activity but that 400 r reduced the degree of enzyme increase that was observed after six or eight daily injections.

4. Injections of 75 mgm./kgm./day of sodium phenobarbital caused a two-fold and a four-fold increase in the enzyme activity of adult female and young male rats respectively. Administration of 600 r to adult females and 400 r to young male rats one day before injection of phenobarbital were begun did not reduce the stimulatory effect of this drug.

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THE EFFECTS OF IONIZING RADIATION ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

III. Further Studies on the Influence of X-Irradiation on the Reductase Activity of the Livers of Rats

Kenneth P. DuBois and Bernard E. Hietbrink

This report concerns: Extension of our previous studies on the influence of radiation on the development of the enzyme system in the liver that catalyzes reductive changes in certain types of chemical agents.

Immediate or ultimate application of the results: Progress in elucidating biochemical effects of radiation requires the systematic investigation of the reactions involved in various phases of intermediary metabolism. As knowledge of previously unknown metabolic reactions progresses to the stage of quantitative measurement of the reactions, it is considered an important part of the present program to ascertain their susceptibility to ionizing radiations. Thus, since the pathway responsible for the oxidative metabolism of foreign chemicals is understood to a considerable extent at the present time, it was of interest to ascertain whether radiation affects the activity of this system. Previous studies in this laboratory demonstrated that the oxidative microsomal enzyme system is unaffected by radiation in adult animals but development of the enzyme system is severely inhibited in young animals. To determine whether this effect occurs generally with respect to microsome enzymes, a study of reductases was undertaken as a step in attempts to locate the exact site of action of radiation. The results of our recent previous experiments and those described in this report indicate a selective action by x-irradiation on the development of oxidative microsomal enzyme systems. Thus it is possible to focus attention on this phase of intermediary metabolism. Elucidation of the exact mechanism responsible for the inhibitory effect of radiation on the development of microsomal enzymes would contribute basic information on a radiation-induced biochemical defect which has not been observed previously.

* * * * * * * *

Previous studies in this laboratory (1-5) demonstrated that the development of enzymes which catalyze the oxidative metabolism of foreign chemicals in the liver is markedly inhibited by sublethal doses of ionizing radiations. In the initial attempts to obtain some information on the specificity of the effect of radiation on microsome enzymes, studies were undertaken (6) to ascertain whether development of the enzyme system that catalyzes reductive changes in chemical agents is similarly inhibited by radiation. The reductase system utilizes reduced triphosphopyridine nucleotide and is present largely in the microsome fraction of the liver (7). A quantitative assay procedure was developed and applied to the livers of normal and irradiated rats (6).

Measurements of the reductase activity of the livers of young rats indicated that the activity is below the adult level at 22 days of age but it increases to the adult level much more rapidly than the oxidative microsomal enzymes (6). Exposure of 23-day old rats to 400 r did not inhibit the rate of development of the enzyme activity. Thus evidence was obtained that radiation has some selectivity in its inhibitory effect on the development of liver microsome enzymes.

The apparent absence of an effect by radiation on the reductase system indicated that reactions which generate the reduced triphosphopyridine nucleotide required for both microsome oxidase and reductase activity are not affected by radiation. Direct evidence in support of this conclusion was obtained by measuring the influence of radiation on dehydrogenases which generate reduced pyridine nucleotides (8,9).

Since the reductase activity of the livers of weanling rats reached the adult level rapidly, it seemed desirable to perform some experiments on hepatectomized rats. This was done with the idea in mind that the reductase activity might be low immediately after hepatectomy and increase at a slow rate during regeneration of the liver as it does in the case of microsome oxidase activity. However, when rats were exposed to 200 r of x-ray at 18 hours following partial hepatectomy no effect by radiation was noted on the reductase activity of the livers at 2-1/4 days and at later intervals after radiation thus indicating that the reductase activity is resistant to radiation.

The present study has consisted of further experiments on the influence of x-irradiation on the reductase activity of the livers of young male rats and partially hepatectomized adult rats. The young animals used for these experiments included some that were less than 22 days of age in order to obtain more definitive information on the influence of radiation given at a time when the enzyme activity was low. Similarly by irradiation of animals and performance of reductase assays shortly after partial hepatectomy it was hoped that further information could be obtained on the effects of radiation on the development of the enzyme activity. The results of these studies provided additional information indicating that the development of reductase activity is not affected by x-irradiation at dosage levels that produce nearly complete inhibition of oxidative microsome enzymes.

Materials and Methods. Young and adult male Sprague-Dawley rats were used for these experiments. The animals were kept in air-conditioned rooms and maintained at 68° to 75° F. and were fed Rockland Rat Diet and water ad libitum.

X-irradiation was administered as single whole body exposures with a G. E. Maximar Therapy Unit. The radiation factors were 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. and the dose rate was 34 r to 36 r per minute.

Reductase activity was measured by the method developed previously in this laboratory (6).

Results

Rate of development of reductase activity in the livers of immature rats. In our previous studies on the reductase activity of the livers of young rats, animals ranging from 22 to 42 days of age were used. However, by 22 days the reductase activity reached about 75% of the adult level. Although the results previously obtained on irradiated animals (6) indicated that radiation does not inhibit the development of this enzyme, it seemed desirable to conduct some further experiments using rats that were less than 23 days old. Prior to the radiation experiments assays were conducted on normal rats at various ages beginning at 14 days of age. The results of these measurements are summarized in Table 1 in which the average and range of values for groups of four rats are presented.

TABLE 1

Rate of Development of Reductase Activity in the Livers of Young Male Rats

Age (Days)	Reductase Activity (ugm. of p-Aminobenzoic Acid/100 mgm of Liver/hr.)			
	Free		Total	
	Average	Range	Average	Range
14	3.8	(3.3-4.0)	11.7	(11.2-12.5)
17	5.5	(5.2-5.9)	14.6	(14.1-15.0)
22	4.9	(4.2-5.5)	23.1	(22.8-24.1)
26	9.6	(6.5-12.3)	32.4	(31.2-33.7)
35	11.5	(11.1-12.0)	35.1	(33.2-36.5)
42	8.9	(8.9-9.3)	30.5	(29.0-32.0)

Reductase assays conducted on the livers of 14-day old rats demonstrated that the enzyme activity is about 1/3 of the normal adult level at that age. The activity increased to about 3/4 of the adult level by 22 days of age and to the adult level by 26 days of age.

Influence of x-irradiation on the development of reductase activity in the livers of immature rats. The low enzyme activity in the livers of

animals at less than 20 days of age made it possible to obtain more definitive information concerning the effect of radiation on the development of this enzyme than could be obtained on weanling rats. To ascertain the effect of radiation on the development of reductase activity in the livers of immature rats, 400 r of x-ray was administered to groups of four animals and they were sacrificed at various times after x-ray exposure. By comparison of the level of enzyme activity in the livers of irradiated animals and in normal rats of the same age it was possible to determine whether radiation had any appreciable influence on development of this system. The results of these measurements are summarized in Table 2.

TABLE 2

Influence of 400 r of X-ray on the Development of Reductase Activity in the Livers of Young, Male Rats

Age at Time of X-ray (Days)	Age at Time of Sacrifice (Days)	Time of Sacrifice After X-ray (Days)	Reductase Activity (µgm. of p-Aminobenzoic Acid/100 mgm. of Tissue/Hour)			
			Free		Total	
			Average	Range	Average	Range
14	18	4	7.0	(5.9-7.9)	18.8	(18.4-19.8)
14	22	8	2.9	(2.5-3.7)	23.2	(21.8-24.5)
23	26	3	11.0	(8.9-13.6)	31.0	(27.2-35.4)
23	35	12	10.8	(10.1-11.4)	30.8	(28.6-32.5)
23	44	21	11.3	(11.0-11.7)	32.0	(30.3-34.4)

When 14-day old rats were irradiated and assays were conducted four days later, there was no inhibition of the development of the enzyme activity and the level of enzyme activity was in fact somewhat higher than in unirradiated rats of the same age. Animals irradiated at 14 days of age and sacrificed at 22 days of age had exactly the same average reductase activity as unirradiated 22-day old rats. The results of these measurements clearly demonstrated the absence of an effect by radiation on the normal development of reductase activity in the livers of young rats.

Influence of x-irradiation on the reductase activity of regenerating rat liver. In a previous study (10) we carried out some measurements of the

reductase activity of the livers of partially hepatectomized normal and irradiated rats. Although the previous study was incomplete, it suggested that the reductase activity returns to normal rapidly after partial hepatectomy. At the time intervals after irradiation and hepatectomy for which comparable data were obtained there was no difference in the reductase activity of the livers of the two groups. In the present investigation additional experiments were conducted since the need for data obtained at short intervals after irradiation was apparent from the previous experiments. A series of adult, male rats was, therefore, hepatectomized. Some of the animals were exposed to 200 r and others were used as controls. Reductase assays were performed on the livers of groups each containing four animals at various times following radiation and partial hepatectomy. The results of these measurements are summarized in Table 3.

TABLE 3

Effect of X-Irradiation on the Reductase Activity of the Livers of Partially Hepatectomized Rats

Days After Hepatectomy	Dose of X-ray	Days After X-ray	Reductase Activity (γgm. of p-Aminobenzoic Acid/100 mgm. of Tissue/Hour			
			Free		Total	
			Average	Range	Average	Range
1	0	.....	10.9	(10.6-11.1)	26.5	(24.8-28.7)
3	0	.....	4.4	(3.8-4.9)	33.3	(32.2-35.2)
7	0	.....	11.8	(11.0-12.6)	38.1	(37.4-39.3)
9	0	.....	10.6	(10.2-10.8)	32.9	(30.7-34.3)
12	0	.....	8.9	(8.8-9.0)	29.7	(28.7-31.7)
3	200 r	2-1/4	13.1	(11.8-14.1)	30.7	(27.3-32.9)
7	200 r	6	14.1	(11.5-14.8)	34.0	(30.4-37.6)
10	200 r	9	7.3	(6.6-7.6)	31.8	(28.9-35.0)

The data obtained on unirradiated rats after partial hepatectomy demonstrated that the reductase activity approaches the normal adult level at 24 hours

after irradiation and at three days after hepatectomy the enzyme activity had completely reached the adult level. When partially hepatectomized animals were exposed to 200 r at 18 hours after hepatectomy, there was no inhibition of the enzyme activity. However, in view of the rapid rate of return of the enzyme activity in the normal hepatectomized animals, the use of young animals was superior for studying the effects of radiation on the development of this enzyme system.

Discussion

The present study was carried out to extend our previous experiments on the effects of x-irradiation on the development of reductase activity in the livers of rats. Since the activity of this enzyme reaches the adult level at a younger age than do the microsome oxidases (11), the use of rats younger than 23 days of age was necessary. By the use of 14-day old rats it was possible to demonstrate rather conclusively that radiation does not affect development of this microsome enzyme system. On the other hand, partially hepatectomized rats were not suitable for measuring the influence of x-irradiation on development of reductase activity because the activity was almost at the normal adult level at one day after partial hepatectomy.

The results of our studies on the influence of radiation on the development of reductase activity demonstrated that the development of this microsome enzyme system is not susceptible to inhibition by ionizing radiations. It thus appears that the inhibition of a microsome oxidase as observed in other studies in this laboratory (1-5) is due to a specific effect by radiation on some step in the reaction between reduced triphosphopyridine nucleotide and the oxidizable substrate. The absence of effects on the reductase system will serve as an aid in further research aimed at elucidating the mechanism of the radiation-induced defect in microsome oxidases.

Summary

1. The reductase activity of the livers of normal, immature, male rats ranging in age from 14 to 42 days was measured. The results of these assays demonstrated that the enzyme activity was about 1/3 of the adult level at 14 days of age and it reached the adult level by 26 days of age.

2. Exposure of 14-day old rats to 400 r of x-ray did not inhibit the rate of increase of the liver reductase activity to the normal adult level.

3. The reductase activity of regenerating liver was found to be near the normal level at one day after partial hepatectomy. The absence of an appreciable decrease in the enzyme activity of the liver makes the hepatectomized rat unsuitable for studying the influence of radiation on the development of this enzyme.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

I. The Influence of Various Chemical Compounds on Radiation Lethality in Mice

V. Plzak, M. Root and J. Doull

This report concerns: The survival time and mortality of male CF_1 mice treated with various chemical compounds immediately prior to the administration of a lethal dose of whole-body x-irradiation.

Immediate or ultimate application of the results: To find chemical compounds capable of reducing or preventing mortality in x-irradiated animals and to elucidate some of the structure-activity relationships within groups of related chemical protective agents. Although none of the currently available radioprotective agents provide a practical solution to the problem of preventing acute radiation injury because of their toxicity or relative ineffectiveness, the study of these compounds and related derivatives provides the most logical approach to finding compounds with an improved therapeutic index. A better understanding of the precise structural configuration(s) responsible for maximal protective activity with minimal toxicity would also be of considerable value in furthering our knowledge of the basic mechanisms of radiation damage in biological systems.

* * * * * * * * *

During the past three months 36 additional chemical compounds were evaluated for protective activity against the lethal effects of whole-body x-irradiation in mice. Since our current studies on the mechanism of action of PAPP and acetyl-PAPP seem to implicate a quinoid structure as the active metabolite responsible for the protective effect of these phenones (1), it was of interest to test benzoquinone and hydroquinone for radioprotective activity as well as a number of quinoline oxide derivatives. The present report also includes results obtained with several monomethyl and dimethyl arsonates, three related pyrazolidines, a thiocyclohexane, a number of triazoles, and a few compounds which are derivatives of previously tested radioprotective compounds.

Materials and Methods. Adult, male CF_1 Carworth Farms mice were employed for these studies. The compounds were dissolved either in water or in propylene glycol and were administered intraperitoneally with the concentration adjusted so that the animals received no more than 1% of their body weight with each injection. Preliminary toxicity studies were carried out with each compound to determine the maximum amount of each derivative which could be administered to the mice without causing mortality due to the chemical toxicity.

At least two dosage levels of each derivative were employed for the radiation studies and a minimum of ten mice were tested at each dosage level,

The compounds were administered 15 minutes prior to the x-ray exposure which consisted of 700 r of whole-body x-irradiation given as a single exposure. The radiation factors were 250 KVP, 15 ma., target-skin distance 75 cm., added filtration 0.25 mm. copper plus 1.0 mm. aluminum; and the dose rate was 40 r per minute as determined by means of a 100 r Victoreen Ionization chamber in air. Control animals were given comparable amounts of the vehicle and irradiated simultaneously with the treated animals. The mortality in the control and treated mice was followed daily for 30 days after the x-ray exposure or until all of the animals were dead. A detailed description of the irradiation procedure, housing and handling has been included in previous reports (2).

The USAF code letter designation and the source of the compounds included in this study are listed in Table 1.

TABLE 1

Source and USAF Code Number of Compounds Included in This Report

USAF Designation	Source of Compound
EK	Eastman Kodak Company, Rochester, New York
AN	Dr. R. M. Moyerman, Ansul Chemical Company, Marinette, Wisc.
WI	Dr. D. T. Witiak, University of Iowa, Iowa City, Iowa
A	Dr. R. Schock, Abbott Laboratories, North Chicago, Illinois
GE	Dr. M. Weiner, Geigy Chemical Corp., Ardsley, New York
HL	Dr. R. Bagdon, Hoffman LaRoche, Inc., Nutley, New Jersey
SZ	Dr. L. B. Achor, Sandoz Pharmaceuticals, Hanover, New Jersey
ST	Dr. N. W. Standish, The Standard Oil Company, Cleveland, Ohio

Results

Preliminary toxicity studies. In order to determine the maximum safe dose for use in the radiation studies, it was necessary to obtain an approximate LD_{50} for the various compounds. Accordingly, small groups of mice were injected intraperitoneally with increasing dosage levels of each compound, and the resulting mortality was recorded for a period of one week. The results of these toxicity tests are shown in Table 2.

Evaluation of compounds for radioprotective activity. Since the x-ray dose used for these studies usually produces 100% mortality within a period of 14 days, a compound is considered to exhibit significant radioprotective activity if it increases the ST_{50} by over five days or if it permits any of the treated animals to survive for 30 days after the x-ray exposure. The results of the radiation studies may be seen in Table 2. Included are the name, number, and structural formula of each of the compounds, the vehicle used for both the toxicity studies and the radioprotective studies, the increase or decrease (in days)

TABLE 2

Acute Intraperitoneal Toxicity and Radioprotective Activity of Various Chemical Compounds in Male CF_1 Mice

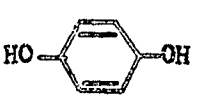
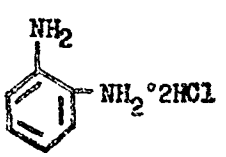
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
p-Benzoquinone EK-P-220 (H_2O) 	5-10	10 5	- 4 0	10/10 10/10
Hydroquinone EK-356 (H_2O) 	100-200	100 50	- 3 0	8/10 10/10
o-Phenylenediamine dihydrochloride EK-678 (H_2O) 	200-300	200 100	- 3 - 1	10/10 10/10

TABLE 2--Continued

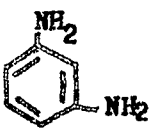
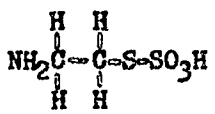
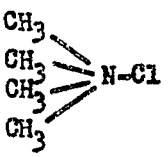
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
m-Phenylenediamine dihydrochloride EK-206 (H_2O) 	100-200	100 50	- 4 0	9/10 10/10
2-Aminoethanethiolsulfuric acid EK-8413 (H_2O) 	300-500	300 100	+ 3 + 3	7/10 9/10
Para-methoxy phenol AN-7 (PG) 	200-300	200 100	- 4 0	9/10 10/10
Tetramethylammonium chloride AN-8 (H_2O) 	25	7.5 5.0	+ 2 + 2	9/10 10/10

Table 2--Continued

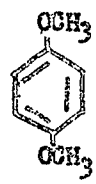
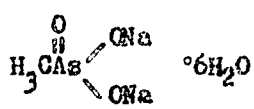
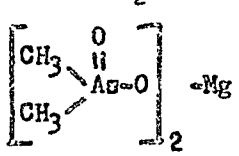
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
p-Dimethoxybenzene AN-9 (FO) 	300-500	300 100	- 7 + 1	10/10 8/10
Disodium methanearsonate $\cdot 6H_2O$ AN-10 (H_2O) 	> 1000	1000 500	- 5 - 2	10/10 8/10
Calcium methanearsonate AN-11 (H_2O) 	500	300 100	- 3 + 1	10/10 10/10
Magnesium cacodylate AN-12 (H_2O) 	500-1000	500 200	- 4 - 3	10/10 10/10

TABLE 2--Continued

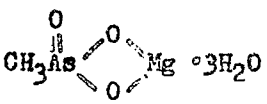
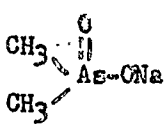
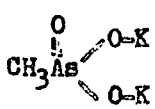
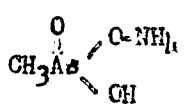
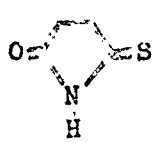
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
Magnesium methanearsonate AN-13 (H_2O) 	> 1000	1000 500	0 - 1	10/10 10/10
Sodium cacodylate AN-14 (H_2O) 	> 1000	1000 500	- 5 - 4	10/10 10/10
Dipotassium methanearsonate AN-15 (H_2O) 	500-1000	500 200	- 4 - 1	10/10 10/10
Monoammonium methanearsonate AN-16 (H_2O) 	> 1000	1000 500	- 3 - 3	10/10 10/10
Monothiosuccinimide WI-1 (PG) 	100-200	100 50	+ 3 +10	6/10 6/10

TABLE 2--Continued

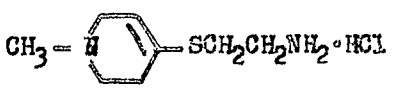
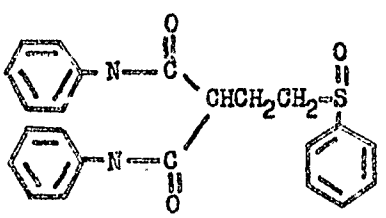
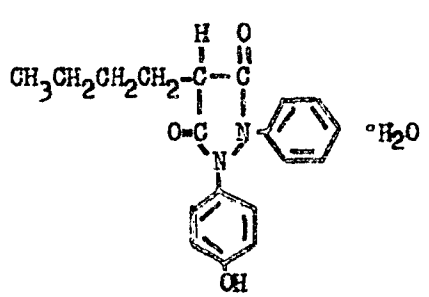
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
1-Methyl-p-aminoethylthiocazo-cyclohexane A-25318 (H_2O) 	200-300	200 100	- 4 0	10/10 10/10
1,2-Diphenyl-4(2'-phenylsulfin-ethyl)-3,5-pyrazolidinedione (Anturane) GE-13 (PG) 	100-200	100 50	0 - 2	8/10 9/10
3,5-Dioxo-1-phenyl-2-p-hydroxy-phenyl-4-n-butyl pyrazolidene (Tandearil) GE-14 (PG) 	100-200	100 50	- 2 0	10/10 9/10

TABLE 2--Continued

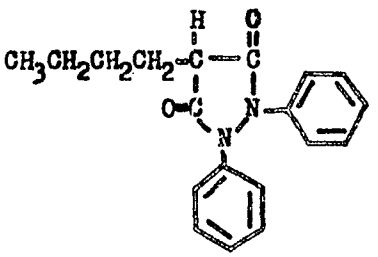
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
3,5-Dioxo-1,2-diphenyl-4-n-butyl-pyrazolidene (Butazolidene) GE-15 (PG) 	100-200	100 50	- 5 0	9/10 9/10
GE-16 (29436) (PG)	100-200	100 50	> + 18 0	2/10 6/10
GE-17 (37665) (PG)	100-200	100 50	+ 4 + 7	6/10 5/10
GE-18 (37367) (PG)	100-200	100 50	- 6 - 1	10/10 10/10
GE-19 (29132) (PG)	200-300	200 100	- 2 + 1	9/10 8/10
GE-20 (37640) (PG)	100-200	100 50	- 1 - 2	10/10 9/10
GE-21 (37641) (PG)	100-200	100 50	- 2 - 3	9/10 10/10
GE-22 (HE-744) (PG)	200	100 50	0 - 3	10/10 9/10

TABLE 2--Continued

Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
GE-23 (HE-101) (PG)	300-500	300 100	- 2 > +18	9/10 4/10
GE-24 (HE-748) (PG)	300-500	300 100	- 4 - 1	10/10 10/10
GE-25 (HE-749) (PG)	300-500	300 100	- 3 + 3	10/10 8/10
GE-26 (HE-750) (PG)	200-300	200 100	- 3 + 4	10/10 7/10
HL-41 (2-5341) (PG)	100-200	100 50	0 - 2	10/10 10/10
HL-40 (2-7561) (PG)	200-300	200 100	- 6 - 1	10/10 10/10
SZ-5 (PG)	100-200	100 50	- 3 - 4	10/10 10/10
ST-15 (2092-2) (PG)	50-100	50 25	- 4 - 1	9/10 9/10
ST-16 (20925) (PG)	200-300	200 100	+13 + 3	5/10 6/10

in the ST_{50} of the treated mice in comparison with that of the simultaneously irradiated controls and the mortality at 30 days after the x-ray exposure.

Benzoquinone and hydroquinone represent respectively the oxidized and reduced forms of the same compound, and since it seems likely from our current studies with PAPP and acetyl-PAPP that a quinoid structure may be the intermediate metabolite responsible for the formation of methemoglobin after the administration of these compounds, it was of interest to test benzoquinone and hydroquinone to see whether they exhibited any radioprotective activity. The latter compound (EK-356), when administered at a dose of 100 mgm./kgm., permitted 20% of the mice to survive the 30-day postirradiation period. Decreasing the dose to 50 mgm./kgm. eliminated the protective effect. Figure 1 shows this effect of hydroquinone on the mortality of mice. In the same figure is shown the protection resulting from the use of 2-aminoethanethiolsulfuric acid (EK-8413) prior to a lethal x-ray exposure. At a dose of 300 mgm./kgm. 30% of the mice survived for 30 days, while at a dose of 100 mgm./kgm. 10% of the mice were protected for the same period of time. The ST_{50} in both instances was increased by three days. Two methoxyphenol derivatives were also studied. When p-methoxyphenol (AN-7) was administered at a dose of 200 mgm./kgm., it afforded only minimal protection (10% of the mice surviving for 30 days), while p-methoxy benzene (AN-9) at a dose of 100 mgm./kgm. protected 20% of the mice for the same period of time. These results may be seen in Figure 2. Several aliphatic compounds, monomethyl and dimethyl arsonates and tetramethylammonium chloride, were included in these tests. Of these the latter, when given at a dose of 7.5 mgm./kgm., was slightly protective as evidenced by a 10% survival of mice for 30 days and disodium methanearsonate (AN-10) protected 20% of the mice at a dosage level of 500 mgm./kgm. Figure 2 shows the protection afforded by AN-10.

Monothiosuccinimide (WI-1) was administered at dosage levels of 100 mgm./kgm. and 50 mgm./kgm. and found to be effective at both doses in protecting mice for 30 days after a lethal exposure to x-ray. Forty per cent of the animals survived in both instances, but at the lower level the ST_{50} was increased by 10 days over that of the simultaneously x-irradiated controls, whereas at the higher level it was increased by only three days. Monothiosuccinimide hydrolyzes to form H_2S and succinimide (3) and the mechanism of the protective action of this compound is presumably related to this chemical degradation. Figure 3 shows the protective effect of WI-1.

Three pyrazolidenes (GE-13, GE-14, GE-15), all diphenyl derivatives and all equally toxic, were evaluated. Minimal radioprotective effects were evident when a dosage level of 50 mgm./kgm. of these compounds was employed with 10% of the mice so pretreated surviving for 30 days after the otherwise lethal x-ray exposure. In addition, when the phenylsulfinethyl derivative (GE-13) was given at a dosage level of 100 mgm./kgm., 20% of the animals survived for 30 days. Figure 3 shows the radioprotective effect of GE-13.

Eleven more compounds, the names and structures of which are not yet released, were also evaluated and of these six were significantly protective (20% or more 30-day survivors), and three exhibited minimal protection (10% survival for the 30-day postirradiation period). Figure 4 shows the protection afforded by the pre-irradiation administration of 100 mgm./kgm. and 50 mgm./kgm. of GE-16 and that afforded by 100 mgm./kgm. and 50 mgm./kgm. of GE-17. The

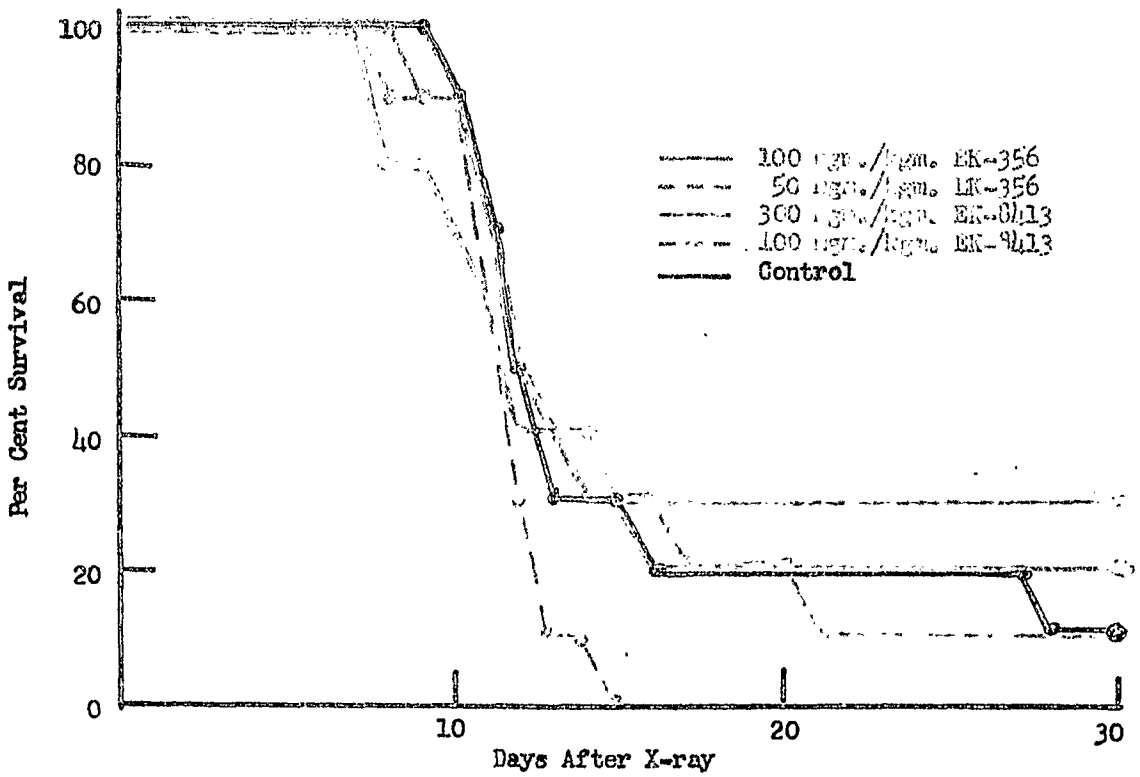


Figure 1. Effect of hydroquinone (EK-356) and 2-aminoethanethiol-sulfuric acid (EK-8413) on survival of mice irradiated with 700 r of whole body x-irradiation.

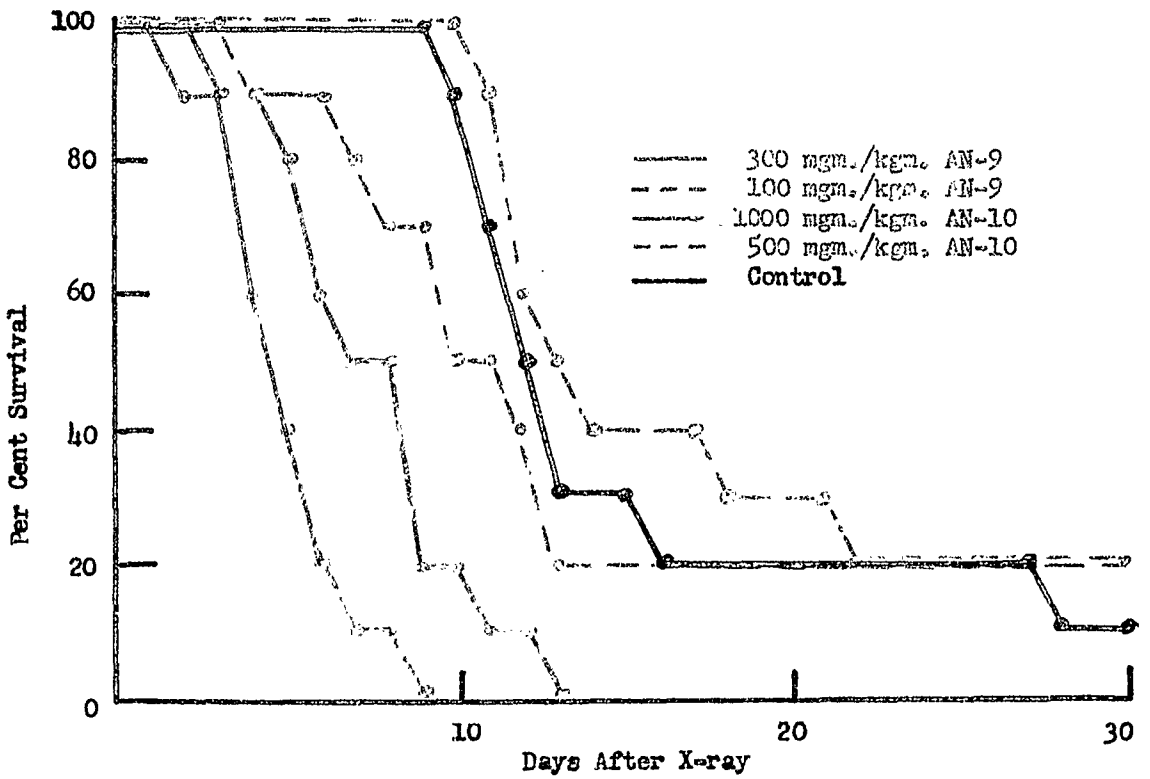


Figure 2. Effect of p-dimethoxy benzene (AN-9) and disodium methanearsonate (AN-10) on survival of mice irradiated with 700 r of whole body x-irradiation.

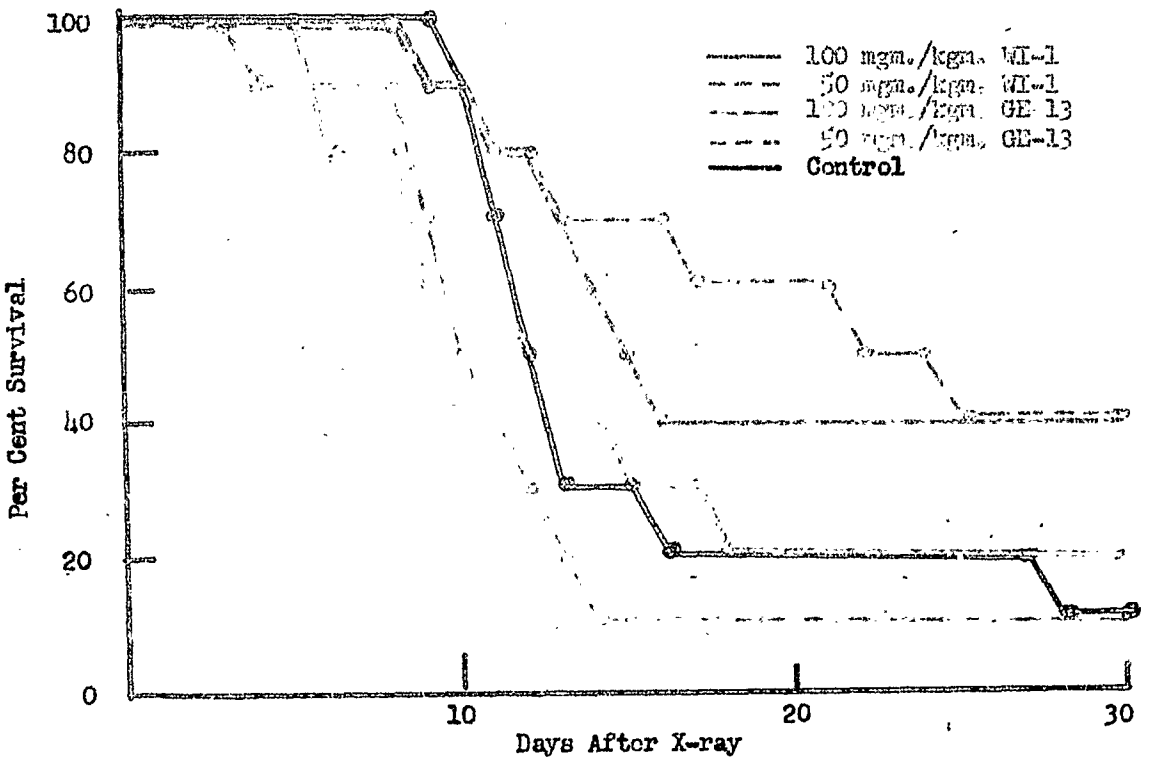


Figure 3. Effect of monothiosuccinimide (WI-1) and 1,2-diphenyl-4(2'-phenylsulfinethyl)-3,5-pyrazolidinedione (GE-13) on survival of mice irradiated with 700 r of whole body x-irradiation.

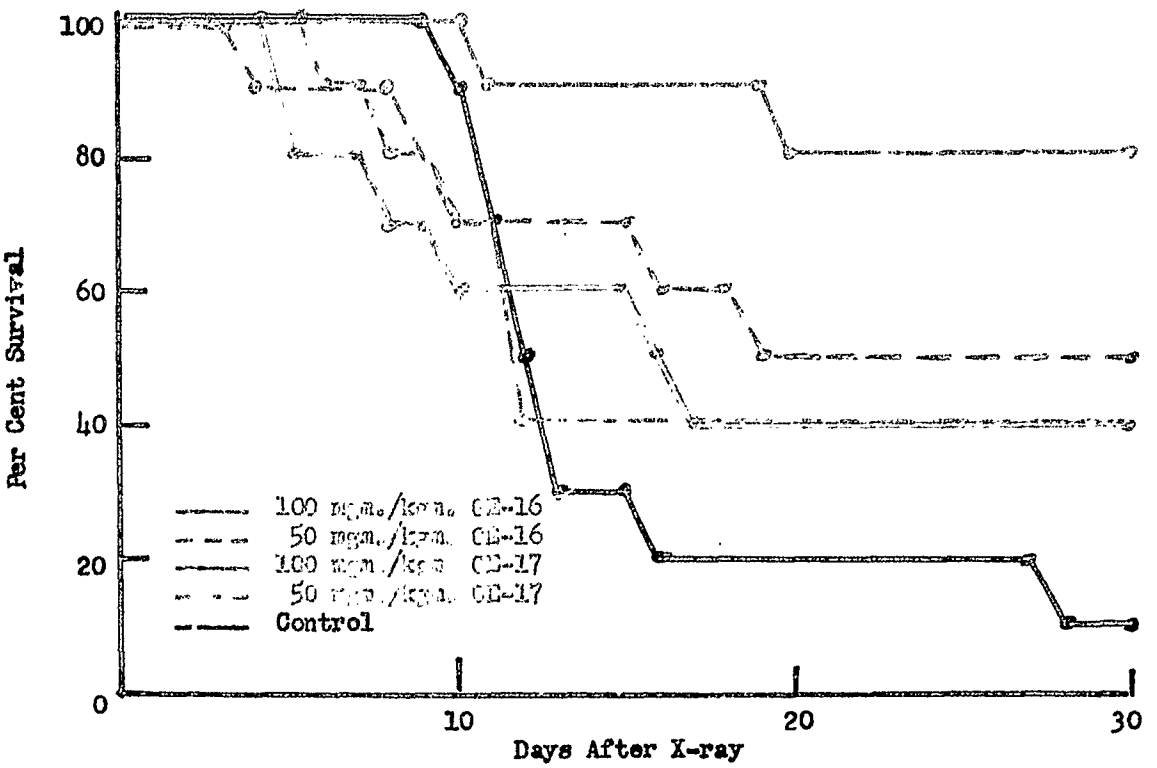


Figure 4. Effect of GE-16 and GE-17 on survival of mice irradiated with 700 r of whole body x-irradiation.

30-day survival was 80% and 40% respectively for the mice given GE-16, while 40% and 50% respectively of the mice given GE-17 survived for 30 days. Figure 5 shows the 30-day survival data for compounds GE-19 and GE-23 where 100 mgm./kgm. of GE-19 permitted 20% of the mice to live for 30 days post-irradiation and GE-23 permitted 60% to survive at the same dosage level. After 200 mgm./kgm. of GE-19, the percentage of survivors was only 10% and 300 mgm./kgm. of GE-23 reduced the protection of that compound to the same level. GE-20, GE-21, and GE-22 protected only 10% of the mice from an otherwise lethal exposure of whole body x-irradiation. When GE-25 and GE-26 were administered at 100 mgm./kgm., 20% and 30% respectively of the animals survived the 30-day period after x-ray. In both instances a higher dose eradicated the protective effects as can be seen in Figure 6.

Five more derivatives of compounds found previously to exhibit varying degrees of radioprotectivity were included of which only two proved to have any value as radioprotective agents. ST-15 protected only 10% of the mice from lethality, but ST-16 was more effective as can be seen in Figure 7. At a dose of 200 mgm./kgm. 50% of the mice survived for 30 days post-irradiation and at a dose of 100 mgm./kgm. 40% survived. At the higher level the ST_{50} was also increased by 13 days over that of the control mice simultaneously irradiated.

Summary

Thirty-six new compounds have been evaluated for protective activity against radiation lethality in CF_1 male mice. Of these 13 were significantly protective in that they permitted 20% or more of the animals to survive an otherwise lethal dose of whole-body x-irradiation. Nine additional compounds protected 10% of the mice for 30 days or more. The best protection was obtained with GE-16 where 80% and 40% respectively of the mice pretreated with two dosage levels of this compound survived for 30 days after the radiation exposure. GE-23 protected 60% at one dosage level and 10% at the other dose employed, whereas GE-17 allowed 40% and 50% of the animals to live for 30 days after x-ray. ST-16, another compound for which a release has not yet been obtained, protected 50% and 40% of the mice at the two dosage levels. WI-1, monothiosuccinimide, was equally protective at both of the doses used, protecting 40% of the animals from lethality. The two compounds which protected 30% of the mice were GE-26 and EK-8413 (2-aminoethanethiolsulfuric acid). The remaining six significant protectors permitted survival of 20% of the mice. They included: EK-356, hydroquinone; AN-9, p-dimethoxy benzene; AN-10, disodium methanearsonate; GE-13, 1,2-diphenyl-4(2'-phenylsulfinethyl)-3,5-pyrazolidinedione; GE-25; and GE-19. Ten per cent of the mice treated with the following compounds also survived for 30 days after irradiation: EK-206, m-phenylenediamine dihydrochloride; AN-7, p-methoxy phenol; AN-8, tetramethylammonium chloride; GE-14, 3,5-dioxo-1-phenyl-2-p-hydroxyphenyl-4-n-butyl pyrazolidene; GE-15, 3,5-dioxo-1,2-diphenyl-4-n-butyl pyrazolidene; GE-20; GE-21; GE-22; and ST-15.

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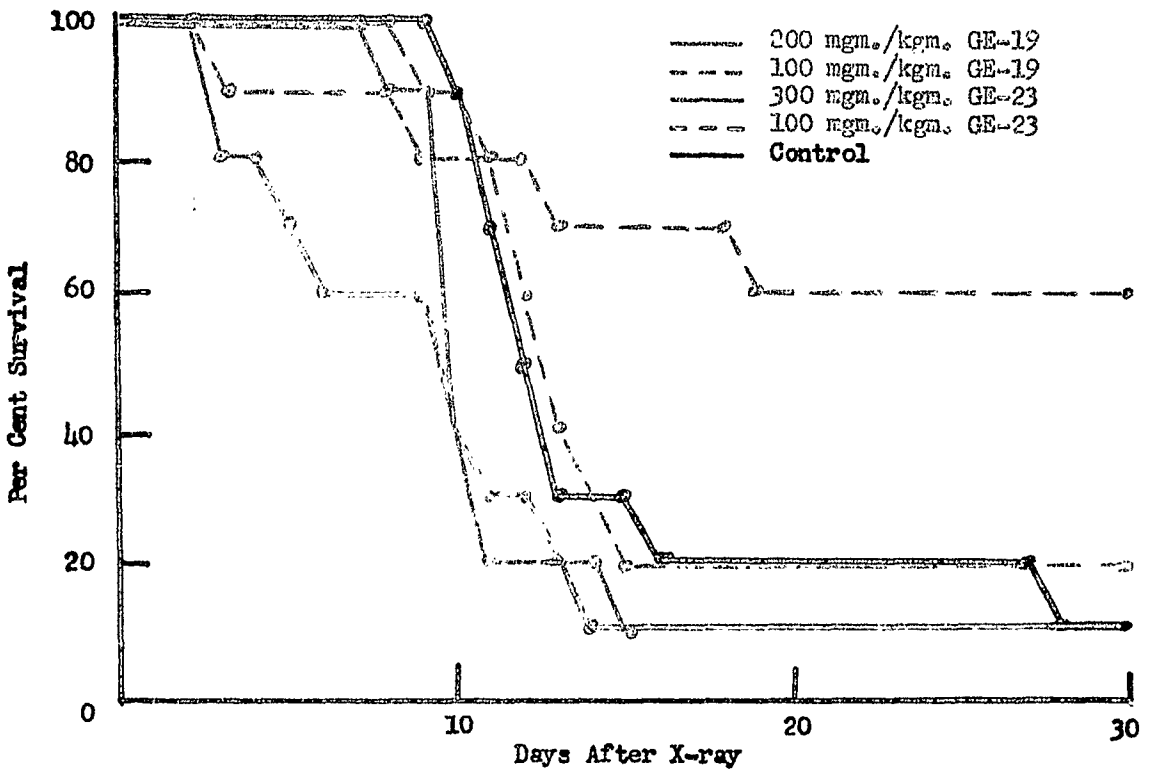

Figure 5. Effect of GE-19 and GE-23 on survival of mice irradiated with 700 r of whole body x-irradiation.

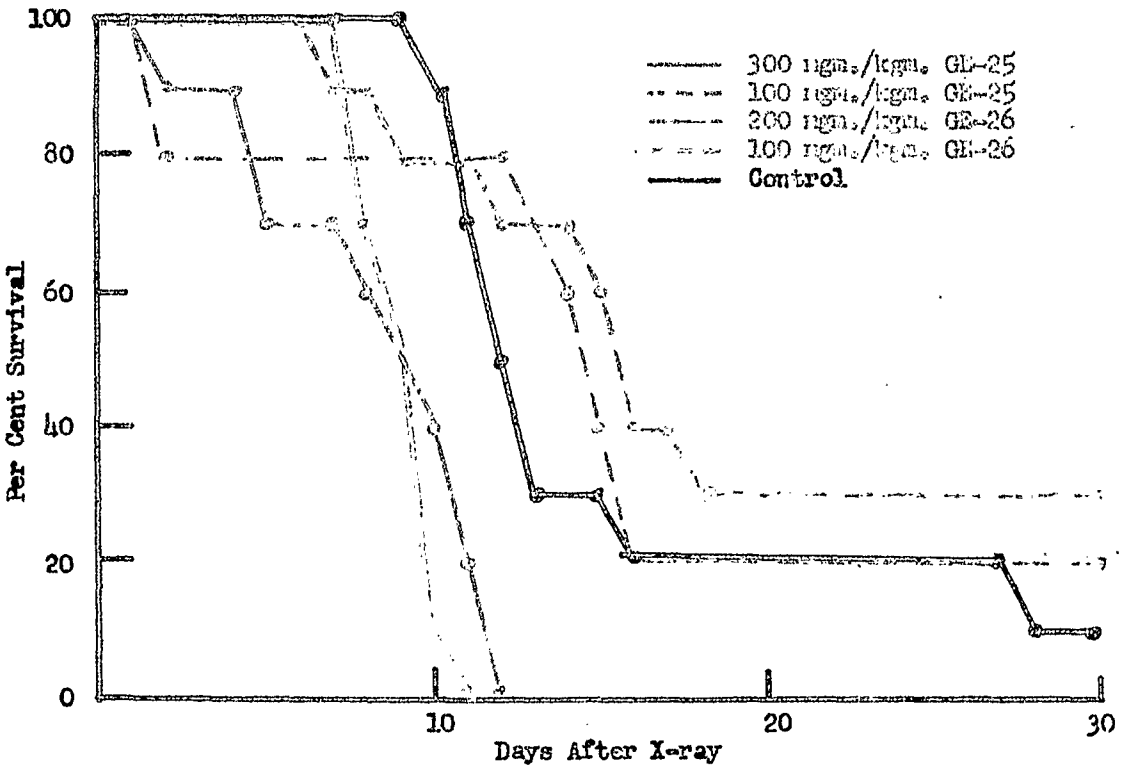

Figure 6. Effect of GE-25 and GE-26 on survival of mice irradiated with 700 r of whole body x-irradiation.

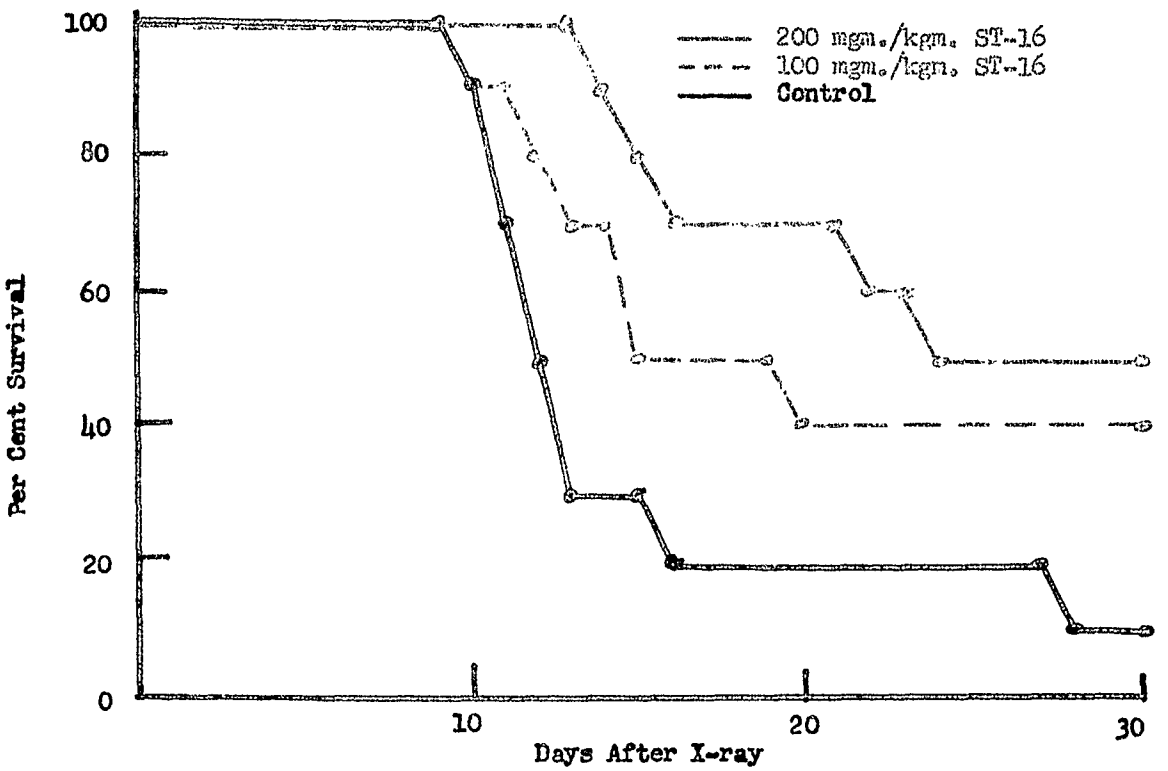

Figure 7. Effect of ST-16 on survival of mice irradiated with 700 r of whole body x-irradiation

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

II. The Effect of Post-Irradiation Administration of Sodium Sulfite and Other Compounds on Radiation Lethality in Female Mice

J. Dilley and J. Doull

This report concerns: The survival time and mortality in female CF_1 mice treated therapeutically with 2-imino-thiazolidine-4-carboxylic acid, sodium sulfite, and sodium sulfite plus l-cysteine after exposure to lethal doses of whole-body x-irradiation.

Immediate or ultimate application of the results: To find compounds capable of reducing radiation injury when given after radiation exposure. In previous studies designed to investigate the mechanism(s) responsible for the radioprotective effect of cyanide and related nitriles, it was observed that cyanide and one of its metabolites (2-imino-thiazolidine-4-carboxylic acid) exhibited therapeutic as well as prophylactic effects against radiation lethality in mice. Although the post-irradiation protective effect of cyanide is slight and that of the metabolite is only moderate, further studies on these and related compounds are indicated because of the lack of agents exhibiting this ability. The development of radioprotective agents which are effective when given after radiation exposure offers in addition to the practical value, a means for increasing our understanding of radiation injury and its prevention and treatment.

* * * * * * * * *

In previous studies (1,2) we demonstrated that the administration of 2-imino-thiazolidine-4-carboxylic acid at 30 minutes after whole-body x-ray exposure will significantly reduce the 30-day mortality in CF_1 female mice. The 2-imino-thiazolidine-4-carboxylic acid used for these studies was an impure preparation obtained by mixing cyanide and l-cystine in a 1 to 2 molar ratio. From additional studies (3) it seems unlikely that the therapeutic effect of this preparation against radiation lethality is due to the original reactants or to other possible reaction products. If the protective effect is due to the thiazolidine derivative, compounds which act at similar sites in the biological system might also be expected to exert therapeutic effects against radiation lethality in mice. The present report contains the results of studies in which sodium sulfite alone and in combination with l-cystine was administered to female mice at 30 minutes after they were given whole-body x-ray exposures.

Materials and Methods. Adult, female Carworth Farms CF_1 mice weighing between 20 and 25 grams were used for these studies. The control and experimental groups were selected from single shipments, housed in groups of not more than eight animals per cage in an air-conditioned room (80° F. $\pm$ 3° F.) and

given food (Rockland Laboratory Chow) and water ad libitum. Aqueous solutions of the compounds were freshly prepared just prior to their use and were injected intraperitoneally in a volume which did not exceed 1% of the body weight of the mice.

The x-ray exposures were given by means of a Keleket X-ray Therapy Unit which was operated at 250 KVP and 15 ma. with 1.0 mm. of aluminum, 0.25 mm. of copper and 1.0 cm. of lucite added filtration. The dose rate was determined prior to each x-ray exposure by means of a 250 r Victoreen Ionization Thimble in air. In order that the x-ray exposure time would be as short as possible, the exposure cage was placed as close to the x-ray tube as was possible without removing the tube shield. Under these conditions the target-skin distance is about 25 cm. and the dose rate is about 235 r per minute. Both the control and experimental groups of mice were irradiated simultaneously and the weight and mortality in each group recorded daily for 30 days after the x-ray exposure or until all of the mice in each group were dead.

Results

Determination of the toxic dose of sodium sulfite in CF_1 female mice. Fresh aqueous solutions of anhydrous sodium sulfite (CP, J. P. Baker Chemical Co., Philadelphia, Pa.) were prepared and injected intraperitoneally into groups of five female CF_1 mice at each dose level. The solutions were prepared so that the total volume injected into each animal did not exceed 1% of the body weight. Mortality observations made daily for a period of ten days indicated that the approximate LD_{50} for sodium sulfite given intraperitoneally to CF_1 female mice is about 900 mgm./kgm.

Survival time and 30-day mortality in CF_1 female mice given sodium sulfite with or without cystine and a partially purified preparation of 2-imino-thiazolidine-4-carboxylic acid at 30 minutes after whole-body x-ray exposures. Since 2-imino-thiazolidine-4-carboxylic acid and sulfite ions undergo many similar reactions with cyanide including an analogous reaction with cystine (4), it was of interest to determine whether sulfite would exhibit therapeutic effects when given to x-rayed mice. In previous studies the maximum therapeutic effect with 2-imino-thiazolidine-4-carboxylic acid was obtained when this compound was given at 30 minutes after the x-ray exposure and a similar interval was, therefore, selected for the present studies with sodium sulfite. Six groups each of which contained 16 female CF_1 mice were used for these studies. Two of these groups were given sodium sulfite (300 mgm./kgm. intraperitoneally) at 30 minutes after the end of the x-ray exposure (600 r whole-body). One group was given 500 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid at 30 minutes after exposure and the fourth group was given 750 mgm./kgm. of the thiazolidine derivative. The fifth group of 16 mice was given sodium sulfite (300 mgm./kgm. intraperitoneally) plus l-cystine (500 mgm./kgm. intraperitoneally), and the sixth group was given comparable amounts of distilled water (controls). The thiazolidine derivative used for these studies was prepared and partially purified using the method of Schoberl and Hamm (5) except that the treatment with copper was replaced by treatment with H_2S to remove excessive lead. Using this modification it

was possible to obtain colorless needle crystals and a light brown granular material which was about 80% soluble in water. A filtered solution of the granular material was used for the present studies in which the concentration of the solution was adjusted so that the animals received about 500 mgm./kgm. of the thiazolidine derivative. The sodium sulfite-cystine solution was prepared by dissolving the cystine in a dilute alkaline solution and adding the sodium sulfite. The solution developed a slightly bluish hue which soon disappeared after which the solution was administered to the irradiated animals.

The results of these studies are summarized in Figure 1. Fifty per cent of the mice treated with the large dose of the thiazolidine derivative (750 mgm./kgm.) survived for 30 days after the x-ray exposure whereas only 25% of the animals given 500 mgm./kgm. of this compound were alive at this time. Eight of the animals in one of the sodium sulfite-treated groups were surviving at the end of the 30-day observation period but there were only three survivors in the other group so that the combined survival in the mice treated with sodium sulfite was about 34% (11 out of 32). Three of the 16 mice given water after the x-ray exposure (controls) also survived for 30 days and there was one animal surviving which had been given sodium sulfite plus cystine. It is evident that sodium sulfite is less effective than the thiazolidine derivative as a therapeutic radioprotective agent when given at 300 mgm./kgm. Additional studies are in progress to determine whether the therapeutic effects of sodium sulfite are improved when this agent is given at different time intervals and to determine whether increasing the dose of sodium sulfite increases the therapeutic effect. The lack of effect when the sodium sulfite is given in combination with cystine is of considerable interest since cystine alone does not markedly alter the 30-day survival of x-irradiated animals (3).

Discussion

The demonstration of a significant protective effect following the post-irradiation administration of sodium sulfite to x-rayed CF_1 female mice is of interest in view of our previous findings concerning the therapeutic effectiveness of a natural metabolite of cyanide, 2-imino-thiazolidine-4-carboxylic acid. Both sulfite and cyanide are known to exert an inhibitory effect on several porphyrin-containing enzymes including the cytochromes, catalases and peroxidases as well as hemoglobin. In many cases this inhibitory activity is readily reversible if cystine is given at the same time or immediately afterward. The fact that in these series of experiments we were able to abolish the protective effects of sodium sulfite by giving cystine at the same time suggests that these particular enzyme systems may be target molecules for damage produced by irradiation. This effect is probably not seen with cyanide since it reacts directly with cystine very quickly, even at room temperature. While sulfite ion undergoes an analogous reaction it takes place much slower.

Recently McLeod *et al.* (6) have described a microsomal, substrate-specific enzyme which they named sulfite oxidase. This is a hemoprotein whose adsorption spectrum in the reduced form resembles that of cytochrome b_5. They found that a variety of molecules including oxygen, cytochrome *c*, and

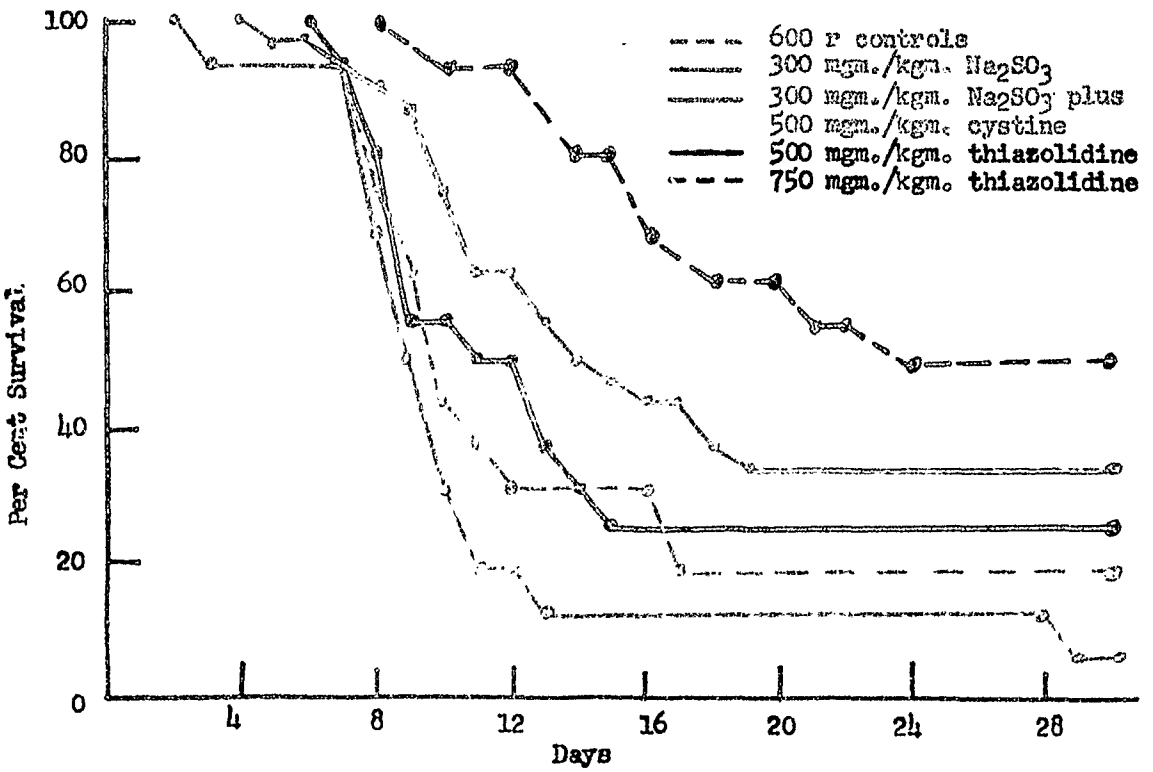

Figure 1. Survival time and mortality in CF_1 female mice treated with various compounds 30 minutes after exposure to 600 r whole-body x-ray. (The numbers in parentheses indicate the number of animals used in each group.)

methylene blue will act as electron acceptors for the reduced enzyme. The reaction which they used to measure the enzyme activity is as follows:

$$SO_3^{=} + H_2O + 2 \text{ ferricytochrome } \underline{c} \longrightarrow SO_4^{=} + 2H^{+} + 2 \text{ ferrocytochrome } \underline{c}$$

Previous studies in this laboratory by Sandberg and Doull (7) have shown that the cytochrome system is slightly inhibited by a single lethal exposure to irradiation in the intact mouse and further studies are planned to see if this effect can be prevented by therapeutic treatment with sulfite.

In addition to the above consideration for the possible mechanism of protection with sulfite, it is of interest to look at its reactions with the molecules which contain disulfide bridges essential for biological activity. Baily and Cole (8) have studied some of these actions in cystine, insulin, trypsin, chymotrypsin, and glutathione and have proposed the following general type reaction:

$$R\text{-}S\text{-}S\text{-}R + SO_3^{=} \longrightarrow R\text{-}S\text{-}SO_3^{-} + R\text{-}S^{-}$$

If disulfide bridges are formed by ionizing radiations as indicated by Barron (9), then the presence of sulfite may be an important factor in the preservation of certain enzyme systems in their active state. Since cyanide has been shown to undergo a similar type reaction (10), further studies of this reaction in normal and x-rayed animals are indicated. Although sulfite was less effective in the present study in preventing radiation lethality in mice than the thiazolidine derivative, it is a more useful agent for investigating such effects since it is easier to obtain, purify, and administer and its reactions are better understood than those of the thiazolidine derivative of cyanide. It is planned, therefore, to extend the present investigation with sulfite to determine the optimal time and dosage level for this agent and to compare its effectiveness against a spectrum of radiation doses.

Summary

The post-irradiation administration of sodium sulfite at a dosage level equivalent to about one-third of the LD_{50} for this agent has been shown to significantly increase the number of animals (CF_1 female mice) surviving at 30 days after 600 r of whole-body x-irradiation. Comparison of the therapeutic effectiveness of sodium sulfite with 2-imino-thiazolidine-4-carboxylic acid indicated that on a molar basis, but not on a mgm./kgm. basis, sodium sulfite is somewhat more effective in preventing radiation lethality in mice. The therapeutic effect of sodium sulfite in x-rayed mice can be eliminated by the simultaneous administration of cystine.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

III. Metabolism and Excretion of p-Aminopropiophenone in Mice

J. Doull and V. Plzak

This report concerns: Measurement of p-aminopropiophenone (PAPP) and its acetyl derivative (acetyl PAPP) in the blood and urine of CF_1 female mice, the conversion of PAPP into a methemoglobin-producing substance and the detection of this substance in the urine of mice given various dosage levels of PAPP or acetyl PAPP.

Immediate or ultimate application of the results: These studies constitute part of a program designed to obtain information concerning the toxic and radioprotective effects of the currently available chemical radioprotective agents. Since the radioprotective effects of p-aminopropiophenone are thought to be due to the production of anoxia in radiosensitive tissues and this effect is attributed to the methemoglobin-producing ability of PAPP, it is of interest to investigate the factors involved in the PAPP-induced methemoglobinemia to determine whether they can be correlated with its radioprotective activity.

* * * * * * * *

In previous studies (1,2) we have investigated the toxicity and radioprotective effects of PAPP and its acetyl derivative in male and female mice and rats. Although the toxicity of PAPP to male rats and mice is similar, female mice are more resistant and female rats are more susceptible to the toxic effects of this agent than males. The toxic effects of PAPP are not directly related to the radioprotective activity of this compound since both male and female rats and mice exhibit comparable protective effects when given the same dose of PAPP prior to whole-body x-ray exposure. Furthermore, the radioprotective activity of both PAPP and acetyl PAPP appears to be relatively independent of the administered dosage level providing the threshold is exceeded. In view of these findings, it was of interest to determine whether the toxic or the radioprotective effects of PAPP (or perhaps both effects) can be correlated with the ability of this agent to produce methemoglobinemia in rats and mice. Both PAPP and acetyl PAPP produce delayed toxic effects in rats and mice and it is difficult to attribute these effects to the transient methemoglobinemia produced by these compounds. However, animals which are treated with either compound frequently die when placed under conditions which increase their respiratory needs (hypoxia, increased environmental temperature, etc.) suggesting that the methemoglobin-induced anoxia of PAPP is responsible for the acute toxic effects of this agent. Similarly, the radioprotective effects of these agents do not correlate well with their methemoglobin-producing effects. Although the time of onset of the methemoglobinemia and radioprotective effect is similar in mice given PAPP, the protective effect precedes the methemoglobinemia in mice treated with acetyl PAPP. The duration of the methemoglobinemia in

PAPP-treated mice is also much longer (three to four hours) than the protective effect in these animals (less than one hour) whereas the duration of the two effects is more closely correlated in mice given acetyl PAPP. Methylene blue which reduces methemoglobin formation in both rats and mice also decreases radioprotective effects but high pressure oxygen exposure which increases methemoglobinemia eliminates the radioprotective effects of the agents.

In additional studies we have also demonstrated that both PAPP and acetyl PAPP must undergo an in vivo conversion since neither agent converts hemoglobin to methemoglobin in vitro. This conversion occurs primarily in the kidneys although other tissues (liver, spleen, heart) are capable of converting PAPP into the methemoglobin-producing agent. It was of interest, therefore, to determine whether the methemoglobin-producing substance could be detected in the urine and whether the urinary level could be related to the urinary or blood levels of PAPP. It was also of interest to follow the urinary excretion of PAPP and to determine whether this agent is acetylated prior to excretion.

Materials and Methods. Adult, female Carworth Farms CF_1 mice weighing between 18 and 22 grams were employed for these studies. The animals were given food (Rockland Laboratory Food) and water ad libitum prior to the urine collection periods but were given only water during the 24-hour period in which they were in the urine collection apparatus. The animals were caged in groups of ten mice during the urine collection period and the urine was collected hourly using a fraction collector. The solutions of PAPP and acetyl PAPP were prepared freshly using propylene glycol as the vehicle and were given intraperitoneally immediately prior to the urine collection periods. The concentration of the injection solutions was adjusted so that none of the mice were given over 1% of their body weight. The control groups of mice were given comparable amounts of propylene glycol.

The urine samples were assayed for methemoglobin-producing substance by adding 0.25 to 1.5 ml. of urine to 0.2 ml. of freshly-drawn mouse blood, incubating for 30 minutes and then determining the conversion of hemoglobin to methemoglobin using the Evelyn Malloy method (3). The Bratton-Marshall method for sulfanilamides (4) was used to determine the urinary content of PAPP and its acetyl derivative. Since this method depends on the diazotization of a free amino group on a benzene ring, it seemed likely that it might be satisfactory for the measurement of both PAPP and acetyl PAPP.

Results

Evaluation of the Bratton-Marshall sulfanilamide method for the measurement of PAPP and acetyl PAPP in the blood and urine of CF_1 female mice. To determine whether the Bratton-Marshall method would be suitable for investigating the distribution and excretion of PAPP, it was necessary to determine first whether any modification of the method was required and second to determine the sensitivity of the method for PAPP. Standard solutions containing 1 mg. of PAPP or acetyl PAPP were prepared in 50% propylene glycol and further diluted with water to obtain concentrations of 10 to 100 gamma of each agent per ml. Analysis of these solutions using the Bratton-Marshall method indicated that a linear response could be obtained within the concentration range of 1 through 25 gamma using a wave length of 540 mμ. The color which developed with PAPP was more

purple than that which is seen with the sulfanilamides but the use of filters having wave lengths of about 510 mμ or 560 mμ did not markedly improve either the sensitivity or the range of the method. The acetyl derivative of PAPP gave no color when treated in the same manner as PAPP but acidification and boiling of the standard solution for one hour to convert the acetyl derivative to the free amine permitted the measurement of the acetyl derivative without significant loss. Standard solutions of acetyl PAPP which were permitted to stand for periods of 24 to 96 hours prior to analysis were found to exhibit some hydrolysis (10% to 25% in 24 hours) indicating that fresh standards of this agent must be prepared daily for use with this method. When PAPP was added to mouse urine or blood at concentrations of between 10 and 1,000 gamma/ml., it was possible to detect between 96% and 102% of the agent following removal of the proteins with 3% trichloracetic acid. It is apparent from these studies that the Bratton-Marshall method is suitable for the analysis of PAPP and its acetyl derivative in the urine and blood of mice. However, since the method is specific only for the amino group of PAPP, it does not provide information concerning metabolic changes in the rest of the molecule. Additional studies are in progress to develop analytical procedures which can be used to follow the changes in the phenone portion of PAPP and its acetyl derivative.

Urinary excretion of PAPP and acetyl PAPP in CF_1 female mice. Six groups of CF_1 female mice, each of which contained ten animals, were used for these studies. Three of the groups were given 10, 20, or 30 mgm./kgm. of PAPP intraperitoneally and the remaining three groups were given 10, 30, or 60 mgm./kgm. of acetyl PAPP by the same route. The mice used for these studies weighed about 20 grams and they were each given 0.1 ml. of the injection solutions which contained 2, 4, or 6 mgm. of PAPP/ml. or 2, 6, or 12 mgm. of acetyl PAPP/ml. in propylene glycol. After the mice were injected, they were immediately placed in a metabolism cage (at about 9:00 A.M.) and the urine collected for a period of 24 hours. Although the urine samples were taken hourly, many of the samples were pooled because of the small volume of urine produced and the results shown in Table 1 are for pooled samples covering the periods of 0 to 6, 6 to 12, and 12 to 24 hours. The samples were analyzed for free PAPP using the Bratton-Marshall method described in the previous section and for bound or acetylated PAPP by the acidification and heating method described for the analysis of acetylated sulfonamides.

During the first few hours after the administration of either PAPP or acetyl PAPP, the mice were depressed and their urine production was small. The urine output was greatest during the 12 to 24-hour period after the administration of the PAPP or acetyl PAPP. However, in all of the studies, this period occurred during the night when the animals are normally active. Neither the PAPP nor the acetyl PAPP appeared to markedly decrease the 24-hour urinary output since two groups of control mice, given comparable amounts of propylene glycol only, had 24-hour urinary outputs of 14.8 ml. and 17.1 ml. respectively.

The results of the PAPP and acetyl PAPP determinations in the urine samples of these animals are summarized in Table 1. It is evident that the excretion of PAPP is greatest during the first six hours after the agents are administered. In several of the experiments in which it was possible to obtain urine samples at more frequent intervals during this first 6-hour period, the greatest excretion of PAPP was observed during the 2 to 4-hour period at which time most of the mice were beginning to recover from the depressant effects of

TABLE 1

Urinary Excretion of PAPP and Acetyl PAPP
in CF_1 Female Mice

Administered Dose in mgm./kgm.	Hours of Urine Collection	Urine Volume in ml.	Urinary Recovery (ugm. of PAPP)		
			Free	Bound	Total
10 of PAPP	0 to 6	2.8	98	690	788
	6 to 24	10.0	127	168	295
20 of PAPP	0 to 6	3.5	304	668	972
	6 to 12	3.1	123	137	260
	12 to 24	7.3	181	83	264
30 of PAPP	0 to 6	3.7	482	982	1424
	6 to 12	0.7	148	97	245
	12 to 24	10.5	173	130	303
10 of Acetyl PAPP	0 to 6	2.0	38	487	525
	6 to 24	14.1	190	108	302
30 of Acetyl PAPP	0 to 6	2.3	137	801	938
	6 to 12	2.8	120	152	272
	12 to 24	13.4	209	88	297
60 of Acetyl PAPP	0 to 6	4.8	587	2128	2715
	6 to 12	0.9	53	196	249
	12 to 24	6.5	275	205	580

the agents. In these studies the injection solutions were also used to prepare the standard solutions for the Bratton-Marshall method so that the recovery of injected PAPP or acetyl PAPP could be determined for each excretion group. The total recovery of the injected PAPP in the 24-hour period was 54.2% for the group given 10 mgm. of PAPP/kgm. and was 37.4% and 32.8% for the groups given 20 and 30 mgm. of PAPP/kgm. respectively. If the acetyl PAPP results are corrected for the difference in molecular weight (192 grams acetyl PAPP equivalent to 149 grams of PAPP) then the total urinary recovery of acetyl PAPP in the three groups was 53.0%, 32.2%, and 37.9% in the mice receiving 10, 30 and 60 mgm. acetyl PAPP/kgm. respectively. Thus it is apparent that the most complete recovery of the injected agent is attained when low dosage levels are given. However, even in this situation almost half of the administered material is not accounted for by urinary excretion alone.

A comparison of the free and bound (acetylated) urinary content of PAPP indicates that, in the PAPP-treated mice, during the early period most of the PAPP is excreted in the bound or acetylated form (about 75%) whereas during the later periods there is more free PAPP in the urine than acetylated PAPP. The same pattern can be observed in the animals given acetyl PAPP. The detection of free PAPP in the urine of the mice given acetyl PAPP is of considerable interest since it indicates that the acetyl PAPP is de-acetylated *in vivo* in mice. It is evident, therefore, that both PAPP and its acetyl derivative are excreted in the free and bound forms.

Conversion of PAPP and acetyl PAPP into a methemoglobin-producing substance and its subsequent excretion in the urine of CF_1 female mice. Preliminary studies were carried out to determine whether a methemoglobin-producing substance could be detected in the urine of female mice given either PAPP or acetyl PAPP. For these studies a 24-hour urine sample was collected from groups of ten animals which had received PAPP (30 mgm./kgm. intraperitoneally) or acetyl PAPP (100 mgm./kgm. intraperitoneally) and from a group of control animals which had been given comparable amounts of propylene glycol. Various amounts of the undiluted urine samples were added to 0.2 ml. of freshly drawn mouse blood and after a 60-minute incubation period, the mixture was analyzed for conversion of the hemoglobin to methemoglobin. The results of these studies are summarized in Table 2 where it can be seen that the per cent conversion of hemoglobin to methemoglobin appeared to be related to the amount of urine added and that the control urine did not cause appreciable methemoglobin formation. An additional sample of the control urine to which PAPP had been added (1 mgm./ml.) was also incubated with mouse blood and since there was no methemoglobin formation in this sample, it is evident that the conversion of PAPP into the methemoglobin-producing substance does not occur *in vitro* in urine. It was of interest to determine whether the methemoglobin-producing substance present in the urine was stable and for these studies the urine samples used for the above studies were permitted to stand for periods of 24 or 48 hours (in a refrigerator) after which they were retested for methemoglobin-producing ability. The results of these studies are also shown in Table 2 where it can be seen that there was only a small decrease in the methemoglobin-producing ability of the urine during the two-day period. It is evident, therefore, that the methemoglobin-producing substance which is formed when mice are given either PAPP or acetyl PAPP is excreted in the urine and that the substance is relatively stable when kept cold. Additional studies are in progress to determine whether heat, pH and other factors influence the stability of this material and to determine

TABLE 2

Studies on the Methemoglobin-Producing Ability of Urine from CF_1 Female Mice Given PAPP, Acetyl PAPP or Propylene Glycol (Controls)

Source of Urine	Incubation Mixture			Per Cent Conversion of Hemoglobin to Methemoglobin
	Urine ml.	Water ml.	Blood ml.	
10 mice given propylene glycol	0.5	1.3	0.2	2.1
	1.0	0.8	0.2	1.9
	1.5	0.3	0.2	2.6
10 mice given PAPP (30 mgm./kgm.)	0.5	1.3	0.2	13.9
	1.0	0.8	0.2	18.5
	1.5	0.3	0.2	44.7
10 mice given Acetyl PAPP (100 mgm./kgm.)	0.5	1.3	0.2	23.6
	1.0	0.8	0.2	44.9
	1.5	0.3	0.2	73.7
Control urine plus PAPP (1 mgm./ml.)	0.5	1.3	0.2	0.0
	1.0	0.8	0.2	1.0
	1.5	0.8	0.2	0.0
Urine from PAPP-treated mice stored for 24 hours at 5° C.	0.5	1.3	0.2	12.6
	1.0	0.8	0.2	20.1
Urine from PAPP-treated mice stored for 48 hours at 5° C.	0.5	1.3	0.2	10.4
	1.0	0.8	0.2	16.8

whether it is as effective in producing methemoglobin in the blood of other species as it is in mice. Studies are also in progress to determine whether the concentration of the methemoglobin-producing substance can be expressed in terms of nitrite equivalents since no standard is as yet available for the material which is derived from the PAPP and acetyl PAPP.

Since the preliminary studies demonstrated that it is possible to detect a methemoglobin-forming substance in the urine of mice given PAPP or acetyl PAPP, it was of interest to determine whether the presence of this material is correlated with the urinary levels of PAPP or acetyl PAPP. Methemoglobin determinations were, therefore, carried out on several of the urine samples used for the PAPP and acetyl PAPP excretion studies. The results of these studies are summarized in Table 3. It can be seen that there was no detectable methemoglobin producing substance in the urine of the mice given the low dose (10 mgm./kgm. intraperitoneally) of either PAPP or acetyl PAPP. When the dosage level was increased to 20 mgm./kgm. of PAPP and 30 mgm./kgm. of acetyl PAPP, the methemoglobin producing substance was detectable during the first six-hour period of urine collection and also during the second six-hour period in the mice given the PAPP. There was no methemoglobin-producing substance in the urine of the mice obtained during the 12 to 24-hour collection period. However, in the animals which received the two highest dosage levels of PAPP and acetyl PAPP, the greatest amount of methemoglobin-producing material was found in the urine which was collected during the 12 to 24-hour period. It is likely that this is due to the fact that these mice produced little urine during the first 12 hours after they had been given the PAPP and acetyl PAPP but additional studies will be required to determine the relationship between urine volume and excretion of the methemoglobin-producing substance.

Discussion

In previous studies (1,2) we have demonstrated that the methemoglobinemia seen in mice given PAPP occurs shortly after its administration and persists for several hours depending on the dose administered. The initial level of methemoglobinemia in such animals is relatively independent of the dose of PAPP, however, within a range of doses of 10 to 60 mgm./kgm. intraperitoneally. The onset of methemoglobinemia in mice given acetyl PAPP is slower and the effect persists for a longer period although the level of methemoglobinemia is comparable to that seen in PAPP treated animals. In mice which have had most of the liver removed, there is a marked delay in the onset of the acetyl PAPP-induced methemoglobinemia but not in that which is produced by PAPP. From these studies we have suggested that acetyl PAPP must first be converted to PAPP (in the liver) and that the methemoglobinemia then results from the subsequent conversion of PAPP to a methemoglobin-producing substance (mostly in the kidneys). The demonstration in the present studies that free PAPP is excreted following the administration of acetyl PAPP supports this hypothesis and the further finding that PAPP is excreted in both the free and bound forms suggests that there is an equilibrium between PAPP and acetyl PAPP in vivo. These studies indicate that both PAPP and acetyl PAPP are rapidly excreted (mostly during the first few hours after administration) in the urine although less than half of the injected material was accounted for in either the free or acetylated forms in the urine during the first day after administration.

TABLE 3

Excretion of a Methemoglobin-Producing Substance in the Urine of CF_1 Female Mice Given Various Doses of PAPP or Acetyl PAPP

Administered Dose in mgm./kgm.	Hours of Urine Collection	Aliquot of Urine Used for Test (ml.)	Per Cent Methemoglobin Formation
10 of PAPP	0 to 6	0.5	0
		1.0	0
	6 to 24	0.5	0
		1.0	0
20 of PAPP	0 to 6	0.5	11.4
		1.0	22.6
		1.5	50.5
	6 to 12	0.5	6.8
		1.0	11.6
	12 to 24	0.5	0
		1.0	0
30 of PAPP	0 to 6	0.5	3.4
		1.0	7.9
		1.5	12.0
	6 to 12	0.5	5.6
		1.0	12.2
		1.5	17.1
	12 to 24	0.5	7.6
		1.0	15.1
		1.5	51.4
10 of acetyl PAPP	0 to 6	0.5	0
		1.0	0
	6 to 24	0.5	0
		1.0	0

TABLE 3--Continued

Administered Dose in mgm./kgm.	Hours of Urine Collection	Aliquot of Urine Used for Test (ml.)	Per Cent Methemoglobin Formation
30 of acetyl PAPP	0 to 6	0.5	1.8
		1.0	3.2
		1.5	4.7
	6 to 12	0.5	0
		1.0	0
		1.5	0
	12 to 24	0.5	0
		1.0	0
		1.5	0
60 of acetyl PAPP	0 to 6	0.5	3.3
		1.0	7.9
		1.5	17.0
	6 to 12	0.5	2.4
		1.0	12.2
		1.5	19.7
	12 to 24	0.5	13.9
		1.0	18.5
		1.5	44.7

The present studies have demonstrated that a methemoglobin-producing substance can be detected in the urine of mice given either PAPP or acetyl PAPP and that it is not formed when PAPP is added to urine in vitro. The excretion of this material in the urine of mice appears to be related to the urine volume although additional studies are required to characterize the excretion patterns more fully. It would appear, therefore, that this metabolic product of PAPP and acetyl PAPP is excreted in a somewhat different pattern than the original substances and this observation may be useful in characterizing the methemoglobinemia effect of these agents. Since the method used for the detection of PAPP and acetyl PAPP in these studies is also applicable to blood, it should be possible to investigate the time course of both the methemoglobin production and the blood levels of the free and bound PAPP in the same animals.

We have previously suggested that the metabolic product of PAPP which is responsible for the methemoglobin production is a quinoid derivative and that it may be a resonating quinoid. It is possible that this substance might be responsible for the radioprotective effect of PAPP and similar phenones and that it is acting as a free radical scavenger rather than through the methemoglobin-forming mechanism. If this were the case, then the ability to detect this agent in the urine may be of considerable value in investigating its effects and may even provide a source of the material since it appears to be relatively stable. We have been unable up to the present time to find a source of PAPP derivatives of this type although several related quinones have been obtained and are being tested for radioprotective effects in mice. Isolation and identification of the methemoglobin-producing agent from the urine would facilitate further studies and it is planned to carry out such procedures during future work on this problem.

Summary

1. A standard method for the detection of free and bound sulfonamides has been used to investigate the excretion of PAPP and acetyl PAPP in the urine of CF_1 female mice. This method which depends on the diazotization of the free amino group and subsequent coupling with an ethylenediamine derivative was found to be suitable for the measurement of the phenone derivatives in the blood and urine of mice at concentrations of 1 to 25 gamma per ml.

2. Both PAPP and acetyl PAPP are excreted rapidly in the urine of CF_1 female mice when given by intraperitoneal administration although higher dosage levels which depress the animals delay the excretion by this route. With low doses (10 mgm./kgm. intraperitoneally) of these agents about 50% of the administered agent was recovered in the free or acetylated form in the urine. Lower recoveries were obtained when the dosage level was increased. During the early period (0 to 6 hours) after the administration of either PAPP or acetyl PAPP, most of the agent in the urine is present in the acetylated form whereas the reverse situation occurs at later intervals (12 to 24 hours). Acetyl PAPP is excreted in the urine of CF_1 female mice as free PAPP as well as in the acetylated form indicating that this agent undergoes a partial in vivo de-acetylation prior to excretion.

3. A methemoglobin-forming substance was detected in the urine of CF_1 female mice given either PAPP or acetyl PAPP. This substance appeared to be

relatively stable when kept cold. Preliminary studies have been carried out to determine whether the urinary concentration of this material is related to the urinary concentration of either the free or bound forms of PAPP.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA AND FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

I. Current Status of the Chronic Low Level Fast Neutron Irradiation Program

A. Sandberg and J. Doull

This report concerns: Survival data for CF_1 female mice exposed to fractionated chronic fast neutron irradiation at various dose rates. This report contains results obtained during the first 54 weeks of a duration of life radiation exposure program.

Immediate or ultimate application of the results: To obtain additional information on the injury and recovery processes from chronic radiation exposure. From the studies now in progress it will be possible to determine the effects of variation in dose rate on the longevity response to fast neutron irradiation. These studies will permit a comparison of chronic fast neutron irradiation with chronic gamma irradiation both from a quantitative standpoint in which life span shortening is the measured parameter and a qualitative standpoint from the histopathological examination of the tissues of the mice which received irradiation exposures. The survival data obtained in this chronic irradiation program will be of value in testing the various model systems which have been proposed for predicting the effects of chronic irradiation and in the formulation of a mathematical statement so that the environmental hazard associated with low level fast neutron exposure can be determined.

* * * * * * * * *

Preliminary studies concerning the life span shortening effects of chronic fast neutron and chronic gamma irradiation have been carried out in this laboratory to determine the magnitude of response which could be achieved with the present facilities. Reports have been presented concerning the complete survival data for the gamma irradiation program (1) and for two of the fast neutron irradiated groups (2) throughout the entire duration of life study. In the previous studies in this laboratory life span shortening was used as the major index for determining the effects of variation in dose rate, total dose, and exposure pattern (3-4). In the present studies a constant exposure pattern is being employed so that life span shortening as a function of variation in dose rate is the principal effect being investigated. In addition to these groups of animals which are being observed for life span shortening effects of chronic fast neutron irradiation, cages of mice have been placed in each of the various dose groups to allow for serial sacrifice at specified time intervals in order to obtain additional information concerning the pathological changes. In the previous fast neutron irradiation programs carried out in this laboratory histopathological information was obtained only on animals which had died or were sacrificed in a terminal condition and the present serial sacrifice schedule will make it possible to expand these findings with respect to

the time of onset, rate of progression of the changes, and the patterns of response of the different organs observed previously (5). Since large numbers of control animals have been employed with each of the various radiation groups, important survival information concerning the normal life span of the CF_1 female mouse will be obtained. Although there are numerous methods of expressing the effects of chronic irradiation on the life span of animals, this report and the interim reports to date contain the data expressed as per cent survival on a weekly basis and the median survival time of the groups which have exhibited a 100% mortality as well as the per cent life span shortening of the latter groups. When complete mortality data are available for the additional low level chronic fast neutron irradiation groups, a more detailed analysis of the data will be presented as well as the histopathological examination of the tissues of the animals.

Materials and Methods. Adult, female Carworth Farms CF_1 mice were used for these studies. The animals were between the ages of 12 and 16 weeks at the beginning of the exposure period. The animals in the 3.18 rad/day group were placed in the fast neutron facility on July 7, 1962 and irradiation exposures were initiated for the 0.47 and 0.36 ra d/day dose groups on June 18, 1962. Two additional groups of animals at dose rates of 0.24 and 0.13 rad/day were placed in the irradiation facility on December 13, 1962. In order to obtain a dose rate intermediate between the highest dose group and the four lower dose groups additional position shelves were placed in the facility to provide a dose rate of 0.99 rad/day and radiation exposures were initiated in this group on April 6, 1963. All of the animals are housed in groups of eight animals per cage in standard laboratory thin-walled plastic cages (6.5" x 11" x 5.5") containing a layer of crushed clay absorber. They are provided with food (Rockland Laboratory Chow) and water ad libitum. The irradiated animals are housed continuously in the fast neutron irradiation facility of this laboratory and the control animals are kept in an area which closely approximates the environmental conditions in this facility. Since the animals could be continually housed in the fast neutron facility and irradiated while in the cages in which they live, no disturbance by additional handling is necessary except for the daily mortality observations. The temperature in the fast neutron irradiation room and in the control room is thermostatically controlled to $80^\circ \pm 3^\circ$ F.

The chronic fast neutron irradiation exposures are administered by means of a 100 curie plutonium-beryllium source having an average energy of 4.5 MEV. The detailed description of this facility, including the placement of the various groups of mice and dosimetry calculations has been presented in a previous report (6). The daily fast neutron exposures are administered over a 9.7 hour time period between 10:00 P.M. and 8:00 A.M. by means of a timing circuit which activates the source hoisting motor.

Results

Effect of chronic fast neutron irradiation on the life span of CF_1 female mice. The life span shortening effect of chronic fast neutron irradiation is at present being investigated in six groups of mice placed in various positions in the fast neutron facility. Based on the flux calculations the neutron dose for the various groups of cages in the median plane (vertical center of the source) are approximately as follows:

Group A	3.18 rad/10 hr. day
Group B	0.47 rad/10 hr. day
Group C	0.36 rad/10 hr. day
Group D	0.24 rad/10 hr. day
Group E	0.13 rad/10 hr. day
Group F	0.99 rad/10 hr. day

The survival data for the groups receiving 3.18, 0.47, and 0.36 rad/day as well as for the control group are shown in Figure 1. The group which received 3.18 rad/day originally consisted of one hundred animals but nine animals were removed for the study of the effect of fast neutron chronic irradiation on the rate of hair growth and the survival data is based on 91 animals. The group which received approximately 0.47 rad/day (Group B) consisted of 192 animals, five of which were removed for hair growth studies and the survival data is presented on the basis of 187 animals. Group C (0.36 rad/day) consisted originally of 128 animals and the data for this group are presented for 122 animals, the remaining six having been removed for the studies previously mentioned. Group D (0.24 rad/day) consisted of 128 animals and Group E (0.13 rad/day) consisted of 64 mice. Group F (0.99 rad/day) consisted of one hundred animals at the initiation of this experiment. At the present time the life span shortening in the three latter groups (D, E, and F) is insufficient to provide any index of the effects of chronic irradiation on life span shortening and these data will be presented in a later report. The control group for irradiation groups A, B, and C consisted originally of one hundred animals and the data are presented for 92 animals, the remainder having been removed for the hair growth study.

A log probit analysis of the mortality data was used to determine the median survival time of Group A since this is the only group which has exhibited sufficient mortality at the present time to allow this type of analysis to be made. Since the number of survivors in the control group is at present too great to allow a calculation of the median survival time, the per cent life span shortening for Group A is based on the control data from the previous experiment (2). At the initiation of both of these experiments, the mice were of the same age range. For purposes of comparison the results of the log probit analysis of the previous two groups are included with the results of this study and are shown in Table 1.

TABLE 1

Effect of Chronic Fast Neutron Irradiation on the Life Span of CF_1 Female Mice

Daily Dose of Fast Neutron Irradiation (rad)	Median Survival Time in Weeks	Life Span Shortening (% of Control)
0 (controls	53	...
0.24	51	3.7
1.66	41	22.6
3.18	32	39.6

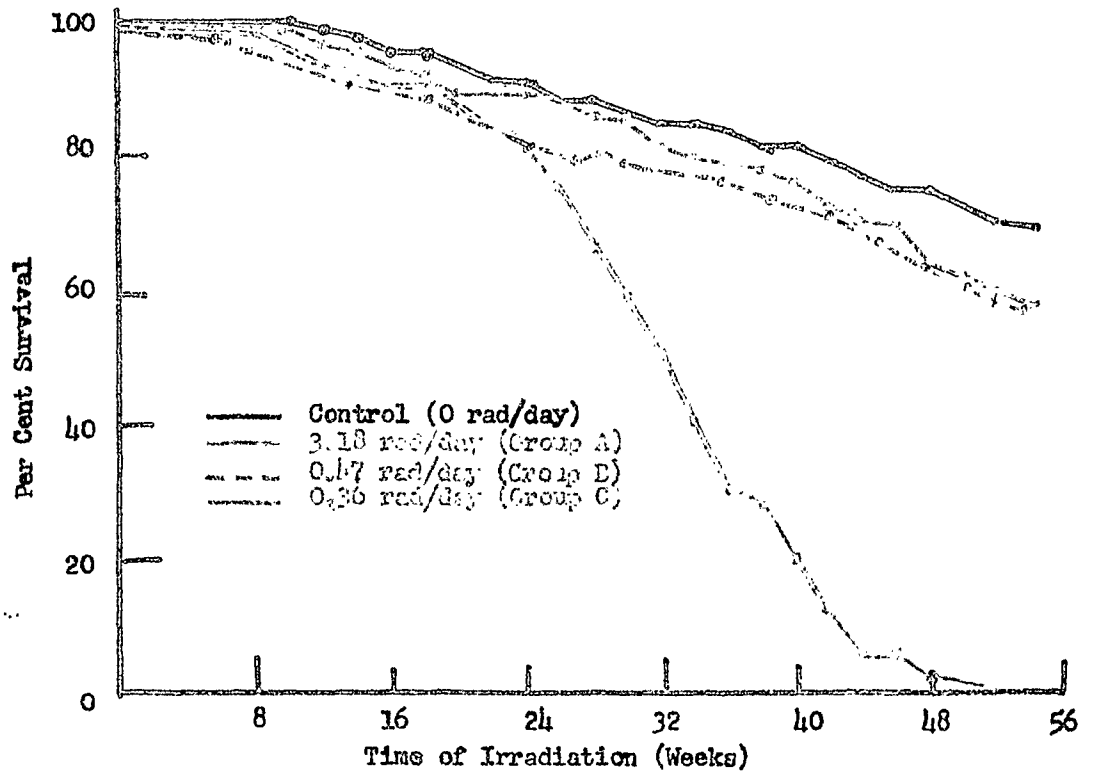

Figure 1. Effect of chronic low level exposure to fast neutron irradiation on the survival of CF_1 female mice.

The probit transformation for this group as well as for the other two groups investigated is shown in Figure 2. As can be seen from these curves, the slopes of the probit analysis curves increase as the radiation dose increases for the two initial groups. However, the slope for that of Group A (3.18 rad/day) is very nearly parallel to that of the group receiving 1.66 rad/day. This would suggest that at the highest dose investigated, the processes leading to death are initiated earlier and accelerated at about the same rate as the processes at a dose level of 1.66 rad/day. When additional data become available concerning the other dose groups under investigation, it will be possible to further determine the effect of dose rate on life span shortening. The histopathological studies will also serve as an index in determining whether or not the causes of death were essentially the same in the control animals as in those receiving daily neutron exposure.

Discussion

Since the preliminary studies in this laboratory indicated that the present neutron irradiation facility is adequate to produce a range of life span shortening of about 0% to 50% by varying the distance of the animals from the source or by increasing or decreasing the period of exposure time, the present program consisting of various dose groups was initiated and has been in progress for 54 weeks.

It has been well established that the LD_{50} for animals receiving chronic irradiation is much greater than that for a single whole body exposure but the actual amount of life span shortening which can be predicted from various chronic irradiation exposures needs to be further investigated. Since the dose rates used in this experiment are of a very low nature, it is anticipated that these data will be useful in determining such effects. In a review of the effects of chronic irradiation on the shortening of life span by chronic gamma and fast neutron irradiation, Mole (7) has presented a compilation of data from several investigators and has stressed the importance of the study of chronic irradiation at low dose levels as well as the type of mathematical analysis applied to these dose levels in an effort to determine whether or not a threshold phenomena exists. Since our present neutron facility permits the study of these low dose levels, the survival data from this program should aid in the interpretation of possible life span shortening in these ranges.

When the present study is completed, we will have complete life span shortening information on eight dose-rate groups at which time it will be possible to attempt a mathematical formulation of the effects of chronic fast neutron irradiation. Melville et al. (8) have mathematically determined the residual damage in animals which have received fractionated radiation doses and since the chronic radiation situation is in reality an extension of the fractionated exposure program, it is planned to attempt to mathematically determine some index of residual damage and the recovery which occurs during the chronic irradiation situation. It is anticipated that the histopathological examination of the tissues of the animals receiving the chronic irradiation exposures will give some indication of the nature of the balance between cellular depletion or damage and the replacement of repair processes. As has been pointed out by Mole (9) an additional factor involved in chronic irradiation

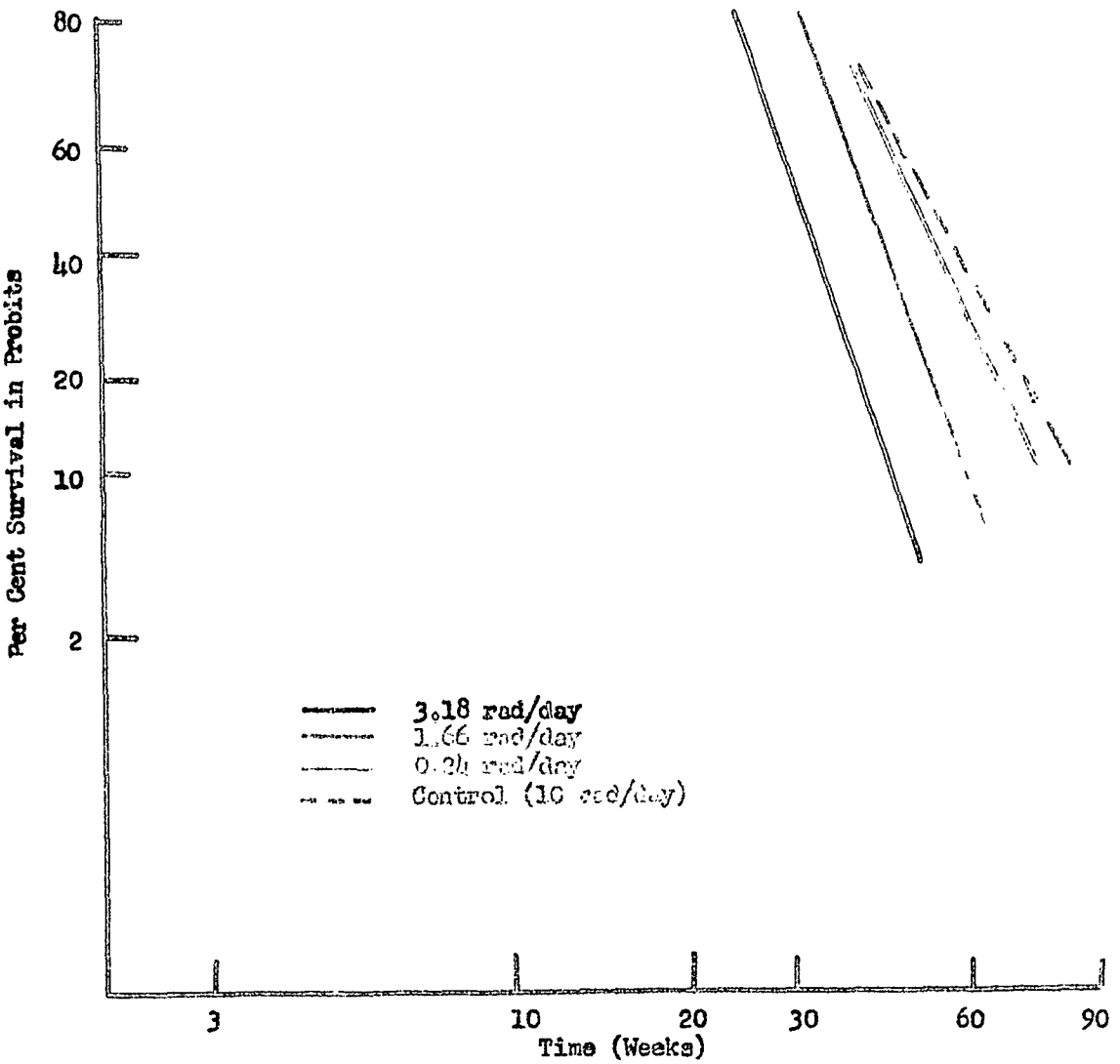

Figure 2. Probit transformation of survival data of CF_1 female mice exposed to chronic fast neutron irradiation.

is that certain specified indices of damage show that adaptation to chronic irradiation occurs.

Our studies have been designed so that the time of exposure for both the chronic gamma and chronic neutron irradiation exposures remains constant eliminating the possible effects of the ratio of radiation time to radiation-free time. In studies in which irradiation was extended from a period of 1.5 to 24 hours with fission neutron and Co^{60} gamma irradiation, Vogel et al. (10) have demonstrated that the dose rate is important in connection with gamma irradiation but that the $LD_{50(30)}$ of fission neutrons is independent of dose rate.

The present fast neutron plutonium-beryllium source has made it unnecessary to adjust the cage positions to correct for the decay of the source and the fact that the present source is an in-line source permits the use of large exposure groups.

Although the two lower dose levels presented in this report have not as yet exhibited a life span shortening of 50%, it is of interest to note that those in the higher of these two groups appear to have an earlier onset of deaths but as the survival approaches 50% the difference between the two groups becomes less marked. The control group for this experiment has not as yet reached a level of 50% mortality so that the per cent life span shortening of the high level group (3.18 rad/day) of chronic fast neutron irradiation cannot be determined. However, since large numbers of control animals have been maintained for the other chronic irradiation studies, an estimate of the life span shortening can be determined and on the basis of this comparison it is 39.6%. When further data concerning the effects of the lower levels of fast neutron irradiation on the survival of these groups becomes available it will be possible to determine more precisely whether the life span shortening as a function of the log of the daily dose is a linear or curvilinear response.

In review of the effects of ionizing radiation and aging, Upton (11) has compiled data from several investigators concerning the relationship between life span shortening and the log of the daily dose rate. The data for the dose groups presented in this report agree with those of these investigators.

The final analysis of the data on all dose groups involved in the present study will make it possible to determine the RBE of fast neutron to chronic gamma irradiation. The dosimetry calculations have been discussed in detail in a previous report and the complete dose measurements will be presented in a subsequent report when it has been possible to determine the gamma contaminant. The complete survival data will be presented when this information becomes available. This information both in regard to life span shortening effects of chronic irradiation and histopathological examination will be used to extend the findings of previous studies in this laboratory and in other laboratories.

Summary

1. Mortality data obtained during the first 54 weeks of a duration of life chronic fast neutron irradiation study are presented and compared with previous studies in this laboratory.

2. The median survival time of the mice exposed to 3.18 rad/day was 32 weeks and a life span shortening of 39.6% was calculated on the basis of survival data for CF_1 female mice from a previous study.

3. The slope of the log probit curve for those mice which received 3.18 rad/day is approximately the same as that for the group of mice that received 1.66 rad/day indicating that, at this dose, the processes leading to death are initiated earlier but may proceed at approximately the same rate as those in groups which received 1.66 rad/day.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA OR FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

II. Studies on the Toxicity of Rare Earth Compounds and Their Influence on Radiation Lethality

David W. Bruce and Kenneth P. DuBois

This report concerns: Studies on the acute intravenous toxicity of praseodymium nitrate when administered alone or in combination with whole body x-irradiation.

Immediate or ultimate application of the results: Because of an increase in their industrial utilization, more information is needed on the toxicity of the rare earth metals. Equally important is the problem of determining the effect of simultaneous exposure to ionizing radiations and rare earth compounds that could result from a nuclear reactor accident or atomic detonation. The present investigation is a continuation of studies to obtain information on the potential hazards associated with the rare earth compounds alone and in combination with ionizing radiations.

* * * * * * * * *

Previous studies in this laboratory (1,2) have shown that various rare earth nitrates given orally to rats are only slightly toxic and moderately toxic when given by the intraperitoneal route. Upon intravenous injection as the nitrate salts the light lanthanons (cerium, praseodymium, neodymium, and samarium) were found to be highly toxic to rats and exhibited a sex difference in that they were 7 to 10 times more toxic to female rats (3) than to males. In this regard, the possibility of a sex difference was suggested by the studies of Snyder et al. (4) which showed that 3.5 mgm./kgm. of cerium salt produced a two-fold increase in liver lipids in female and castrated male rats but no significant change in the lipid content of intact males. Snyder and Stephens (5) found that intravenous cerium chloride also caused a decrease in serum glucose of female rats followed by an increase in plasma free fatty acids suggesting that the first effect of cerium chloride was on carbohydrate metabolism. Studies in this laboratory (6) have demonstrated that intravenous administration of 2 mgm./kgm. or 4 mgm./kgm. of praseodymium as the nitrate salt resulted in a proportional decrease with respect to time in the blood glucose of female rats during the 12- to 72-hour period following administration. At any given time during this period, the per cent decrease in blood glucose from control values after 4 mgm./kgm. was twice that seen after the administration of 2 mgm./kgm. By administration of testosterone propionate (5 mgm./kgm.) daily for a period of thirty days prior to 2 mgm./kgm. of praseodymium or by placing female rats on a high carbohydrate diet by giving solutions containing glucose or sucrose, mortalities could be decreased and the decrease norrally seen in blood glucose could be modifed or prevented (7).

Studies by Melville and Riess (8) and studies in this laboratory have also demonstrated an increase in mortality of rats receiving the rare earth salts intraperitoneally in combination with whole body radiation. When sublethal doses of the rare earth nitrates were given with 500 r of x-ray, a 27% to 82% increase in mortality was observed (9). A 34% to 63% increase in the toxicity of intravenously administered praseodymium nitrate (2 mgm./kgm.) was observed when given 10 to 15 minutes prior to doses of whole body x-irradiation ranging from 50 r to 500 r (6).

Materials and Methods. Adult, male and female Sprague-Dawley rats (200 to 270 gm.) were used for these experiments. The animals were housed in air-conditioned quarters and given water and Rockland Rat Diet ad libitum. Aqueous solutions of praseodymium nitrate (pH 3.5-5.5) in isotonic saline were given by tail vein; control animals received an equivalent volume of saline equal to 0.1% of the total body weight. A colloidal hydroxide suspension (pH 7.5) of praseodymium nitrate was prepared for intravenous injection by the addition of sodium carbonate to the dissolved nitrate salt.

Blood glucose (total reducing value) was determined by the method of Folin and Malmros (10) employing the micromodifications of Park and Johnson (11). Serial samples of whole blood (0.05 ml. in duplicate) were obtained by sectioning the tail under local anesthesia. Tissue slices of rat liver were prepared using a Stadie-Riggs microtome (12). The slices were suspended in Krebs-Ringer-phosphate buffer (pH 7.4). The endogenous respiration was measured manometrically at 38° C. in an atmosphere of pure oxygen following a 10-minute equilibration period. The QO_2 values were calculated from the dry weight of the tissue slices which were dried to constant weight at 105° C.

X-irradiation was administered as a single, total-body exposure with a G. E. Maximar therapy unit. The radiation factors were as follows: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target animal distance was 75 cm. and the dose rate was 35 r to 37 r/minute as measured in air with a Victoreen ionization chamber.

The nitrate compound used in this study was obtained from Lindsay Chemical Company, West Chicago, Illinois.

Results

Effect of intravenous praseodymium nitrate on mortality and blood glucose of intact and castrated adult male rats. Because of the sex difference noted in the intravenous toxicity of the light lanthanons and the suggested hormonal involvement, it was of interest to see what effect castration of male rats would have on the toxicity of praseodymium. Male rats 50 days old were castrated using pentobarbital sodium as the anesthetic agent. Fifteen days after castration groups each containing ten animals were injected with 4, 8, and 12 mgm./kgm. of praseodymium as the nitrate salt and observed for a period of 30 days.

The results presented in Table 1 show that 4 mgm./kgm. and 8 mgm./kgm. caused mortality of 40% and 50% of the rats, respectively, while 12 mgm./kgm.

resulted in only a 20% mortality. In previous studies conducted to determine the approximate LD_{50} value for praseodymium in normal males, doses of 6 mgm./kgm. and 12 mgm./kgm. were lethal to only 20% of the animals when given as the nitrate salt. The results suggest that the hormonal factors alone may not be the explanation for the increased toxicity observed in castrated male rats. In addition to further mortality studies in progress to clarify the above results, experiments were conducted to ascertain whether 8 mgm./kgm. of intravenously administered praseodymium had any effect on the blood glucose of castrated and normal male rats. Prior studies have shown that in contrast to the marked decrease in blood glucose of female rats caused by 2 mgm./kgm. of praseodymium, neither 2 mgm./kgm. of praseodymium metal nor equitoxic doses of 20 or 30 mgm./kgm. produced appreciable changes in the blood glucose of male rats.

TABLE 7

Acute Intravenous Toxicity of Praseodymium Nitrate to Castrated Male Rats

Dose (mgm./kgm. Metal)	pH Solution	Mortality[a]	% Mortality
4	5.35	4/10	40
8	5.20	5/10	50
12	5.10	2/10	20

[a] Mortality data based upon 30-day observation period.

Figure 1 shows the effect of 8 mgm./kgm. of praseodymium on the blood glucose of normal and castrated male rats. The values are plotted as per cent of the control blood glucose values. Each animal served as its own control and each point on the curve represents the average value obtained for groups each containing four to eight animals. The control values in each animal were obtained 24 hours prior to praseodymium administration and serial samples were also obtained for control animals at each 24-hour period.

In contrast to results previously obtained with other doses of this compound, a marked decrease in blood glucose in both intact and castrated males was found. An average decrease from control values of 13% and 23% at 24 hours, 34% and 31% at 48 hours, and 41% and 31% at 72 hours was found for intact and castrated males respectively.

In these studies the majority of animals died during the first six days post-injection as previously found in other intravenous toxicity studies with the light lanthanons.

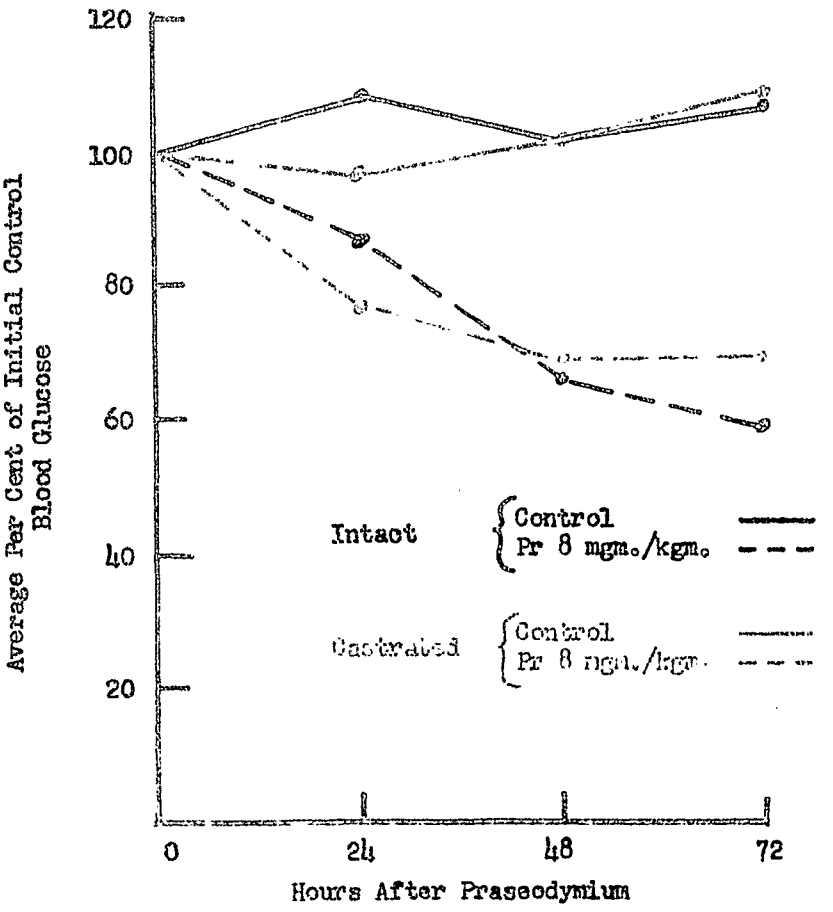

Figure 1. Effect of intravenously administered praseodymium on the blood glucose of castrated and intact adult male rats.

Effect of whole-body x-irradiation on the toxicity of intravenous praseodymium nitrate. In conjunction with the studies with castrated rats, preliminary studies were conducted to observe the effects of administration of 8 mgm./kgm. of praseodymium as the nitrate salt 15 days after a single dose of 400 r of total body x-irradiation. X-irradiation was administered to a group containing ten 50-day old male rats. Ten days after injection this dose of rare earth nitrate was found to be lethal to 90% of the rats. Further studies are being conducted.

Effect of varying the pH of intravenously administered praseodymium on the endogenous respiration of liver slices from female rats. Previous studies (13) have indicated that measurement of the endogenous respiration of liver slices from female rats is an adequate means of determining the toxicity of intravenous praseodymium. Since the pH of the intravenously injected solution may be a factor in the toxicity of the compound, solutions of praseodymium nitrate (2 mgm./kgm. of metal) at pH 3.5 and 5.5 and a colloidal suspension at pH 7.5 were given to groups of rats each containing four animals. Control animals received an equivalent amount of isotonic saline. Forty-eight hours after injection, the animals were sacrificed and the endogenous respiration of liver slices determined. As shown in Table 2, the average QO_2 values obtained on liver slices from rats receiving solutions at pH 3.5 and pH 5.5 were 5.3 or 51% of the control value. The average QO_2 value for liver slices from rats receiving the colloidal suspension was 10.7 and not significantly different from the average control value. The results suggest that it is the ionized salt and not the colloidal suspension that produces the toxic effects seen in female rats.

TABLE 2

Effect of pH of Intravenous Praseodymium on the Endogenous Respiration of Liver Slices from Female Rats

pH Injected Praseodymium	QO_2 Values 48 Hours Post-Injection	% of Control Activity
Saline controls	10.3 (9.0-10.9)	.....
3.5	5.3 (4.2-6.8)	51.4
5.5	5.3 (4.8-6.0)	51.4
7.5	10.7 (10.2-11.1)	104.0

Discussion

Studies on the acute toxicity of intravenous praseodymium nitrate in intact male and female and castrated male rats may indicate that the toxic actions of praseodymium result from more than one mechanism of action. Our results suggest that the physical-chemical state of the intravenously injected solution is an important factor. Although there is a similarity in the distribution of the colloidal and ionic forms of the light lanthanons (14), the toxic action of the two forms may be different. In vivo studies by Aeberhardt (15) with tracer doses of ionic cerium-144 demonstrated that 90% to 95% of the ionic form was bound to the gamma globulins and beta-2 globulins of the plasma proteins in rats, guinea pigs, and rabbits. The remainder was fixed to the white cells. He found that this binding was accomplished before the buffering action of the blood could form colloidal aggregates. Intravenously administered colloidal cerium-144 was found to remain unbound in the blood. Since the light lanthanons accumulate to the greatest extent in the liver (15), the results suggest that one toxic mechanism of action is dependent upon the amount of light lanthanon that is transported to the liver bound to the plasma proteins.

Summary

1. Intravenous administration of 4 mgm./kgm., 8 mgm./kgm., and 12 mgm./kgm. of praseodymium as the nitrate salt resulted in mortality of 40%, 50% and 20% respectively of adult castrated rats that were injected 15 days after castration and observed for a period of 30 days.

2. The intravenous administration of 8 mgm./kgm. of praseodymium as the nitrate salt caused a decrease in blood glucose in both intact and castrated male rats. The average per cent decrease from control values was 13% and 23% at 24 hours, 34% and 31% at 48 hours, and 41% and 31% at 72 hours for intact and castrated males respectively.

3. The administration of 2 mgm./kgm. of praseodymium at pH 3.5 and pH 5.5 resulted in a 51% decrease in the endogenous respiration of liver slices from female rats 48 hours after administration. When 2 mgm./kgm. of praseodymium was given as a colloidal suspension at pH 7.5 the QO_2 values obtained did not differ significantly from control values.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA AND FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

III. Radiation Pathology of Chemically Protected Mice Serially Sacrificed Following Proton or X-irradiation

D. Vesselinovitch, F. Fitch, J. Meskauskas, D. G. Oldfield, V. Plzak and J. Doull

This report concerns: The type and extent of gross and microscopic changes in the tissues of CF_1 male mice given 2-mercaptoethylamine (MEA) prior to whole-body irradiation with 440 Mev protons or 250 Kvp x-rays and sacrificed daily from 1/2 through 15 days.

Immediate or ultimate application of the results: Information is needed concerning the relative biological effectiveness of high-energy protons in mammalian systems and the ability of various environmental factors to modify the injury produced by this type of radiation exposure. The studies described in this report constitute part of a program designed to provide information on the biological effects of high-energy proton irradiation, protection against these effects by means of chemical radioprotective agents, and a comparison with results obtained using radiations having other LET values (10 to 50 Mev electrons, relativistic neutrons, lower energy protons, etc.). In addition to the practical values of such studies, they are also of theoretical interest with regard to the relationship of LET to radiation injury and to the mechanism of action of the chemical radioprotective agents.

* * * * * * * *

In a previous report (1) we have described the terminal gross and microscopic pathology in mice which died as a result of exposure to proton or x-irradiation and the influence of pretreatment with chemical radioprotective agents on these effects. The present study was undertaken to investigate whether significant differences exist in the nature or time-course of radiation-induced injury and recovery processes in proton-irradiated as compared with x-irradiated mice. It was also of interest to determine whether these processes would exhibit different temporal patterns when the irradiation was preceded by treatment with a protective substance. Because of its importance as a protective agent, and to facilitate comparison with the proton and x-ray protection studies already available in this laboratory, MEA was selected as the protective agent for these studies. The radiation doses were chosen to lie in the neighborhood of the $LD_{50/30}$ for each of the two types of radiation. Thus the basis for comparison of the unprotected proton with the unprotected x-ray groups of mice is the equilikelihood of survival to 30 days for the two groups.

Materials and Methods. Male CF_1 mice were used for this study. The mice were 17 to 18 weeks old at the time of irradiation. The animals were selected from several shipments, randomized, and assigned to various protected, unprotected and control groups. A detailed description of the physical and

biological methods used in the irradiation of the animals has been presented in a previous report (2). Table 1 presents the experimental design showing the time of serial sacrifice, the various dose groups and the number of animals examined in each group. In addition to the tabular listing, five unirradiated, untreated animals were taken as controls. Only living mice were taken for examination of the tissues and these animals were sacrificed under ether anesthesia. The following tissues were routinely taken for histological examination: liver, kidney, spleen, heart, lungs, thymus, testes, lymph nodes (mediastinal and mesenteric), duodenum, pancreas, and sternum. The tissues were fixed in neutral buffered formalin, embedded in paraffin, and stained routinely with hematoxylin and eosin. Most of the sections of the spleen and some of the bone marrow sections were stained with Azure-Eosinate. Spleen imprints were made from all animals examined and stained with Wright-Giemsa stain. A few sections of the liver were stained with Trichrome-Gomori Aldehyde Fuchsin. In the case of spleen, the wet weight was obtained immediately upon sacrifice and prior to any histological processing.

Results

Gross pathological findings. Table 2 presents a summary of the major gross pathological findings in proton-irradiated mice. The most frequent pathological finding at postmortem examination was hemorrhage in the form of petechiae or ecchymosis involving areas of various size in the affected organs. The organs so involved were brain, liver, and subcutaneous tissues in the abdominal region. Another interesting finding was appearance of pin-point nodules scattered predominantly through the red pulp of the spleen. Slightly enlarged mesenteric lymph nodes were observed in one proton-irradiated mouse and in a few animals pretreated with MEA. There was a soft stone in the urinary bladders of a few mice given protons with and without MEA treatment. Patchy pneumonic consolidation of the lungs was seen in a few experimental mice regardless of the treatment given.

In general, no distinctive temporal pattern of progression or retrogression of these lesions emerged from the gross, qualitative, pathologic study of proton-irradiated animals. A similar result was obtained for the x-irradiated mice and will not be reported here in detail.

Data on spleen weights found at autopsy are given in Table 3 and are plotted in Figures 1 to 4. From these data it can be seen that the pattern of spleen weight change after irradiation is similar in all irradiated groups. The weight decreases to a minimum during the first 72 hours after irradiation and remains at this minimum during the next 72 hours. Recovery from the weight loss begins on the sixth to seventh day. There is some indication from the curves that in both the proton and x-ray groups, the spleen weight minimum observed during the second 72 hours is more severe, i.e., the weight is lower in unprotected than in protected groups.

Microscopic findings. The major histopathological findings in the tissues of animals examined in these studies are summarized in Tables 4 and 5. The major findings were seen in the spleen, thymus, lymph nodes, bone marrow, testis, liver, and brain.

TABLE 1

Number of Animals Examined at Each Assay Point

Time of Sacrifice (days)	Radiation Dose and Pre-irradiation Treatment			
	1/2% Body Weight H_2O		225 mgm./kgm. MEA	
	707 rads Proton	580 rads X-ray	707 rads Proton	580 rads X-ray
1/2	6	...	3	...
1	6	4	3	4
2	6	4	3	4
3	6	4	3	4
4	6	4	3	4
5	6	4	3	4
6	6	4	3	3
7	6	4	3	3
8	6	4	3	3
9	6		3	
10	4		3	
11	4		3	
12	4		3	
13	4		3	
14	4		3	
15	3		3	

TABLE 2

Frequency and Severity of Major Gross Pathologic Findings in Proton-Irradiated Mice

Major Gross Pathologic Findings	Treatment	Time of Serial Sacrifice (Days Post-irradiation)															
		1/2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Hemorrhages in the brain	None	...	...	...	...	...	...	...	...	...	...	...	0/5	...	...	...	...
	H_2O	0/6	0/6	0/6	3/6 a_2a_2a	0/6	0/6	0/6	0/6	0/6	0/6	0/4	0/4	0/4	0/4	0/4	0/3
	MEA	0/3	0/3	1/3 a	1/3 a	0/3	0/3	0/3	0/3	0/3	0/3	2/3 a_2a	0/3	0/3	1/3 a	0/3	0/3
Spotty hemorrhages in the liver	None	...	...	...	...	...	...	...	...	...	...	...	0/5	...	...	...	...
	H_2O	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/4	1/4 b	0/4	0/4	0/4	0/3
	MEA	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 a	0/3	0/3
Nodular spleen	None	...	...	...	...	...	...	...	...	...	...	...	0/5	...	...	...	...
	H_2O	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6 a	0/4	2/4 a_2b	0/4	0/4	0/4	0/3
	MEA	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 b	0/3	0/3	0/3	0/3
Enlarged mesenteric lymph nodes	None	...	...	...	...	...	...	...	...	...	...	...	0/5	...	...	...	...
	H_2O	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/4	0/4	0/4	0/4	0/4	0/3
	MEA	0/3	0/3	0/3	0/3	1/3 a	1/3 a	1/3 a	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Miscellaneous (soft stones)	H_2O	0/6	1/6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/4	0/4	4/4	0/4	0/4	0/3
	MEA	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3

TABLE 3

Spleen Weight as a Function of Time of Sacrifice
in the Irradiated Groups

t, Days Post-irradiation	707 rads 440 Mev Protons				580 rads 250 Kvp X-rays			
	H_2O		MEA		H_2O		MEA	
	m (mgm.)	± s	m (mgm.)	± s	m (mgm.)	± s	m (mgm.)	± s
1/2	59	± 4	49	± 9				
1	67	3	67	4	67	± 4	69	± 3
2	44	5	53	9	59	7	86	20
3	41	3	60	4	46	5	66	5
4	43	7	47	10	51	5	64	2
5	58	9	57	10	52	7	50	11
6	41	7	102	10	26	2	49	4
7	54	3	61	7	45	2	55	4
8	39	5	62	10	79	35	76	15
9	68	14	103	31				
10	80	16	64	13				
11	74	14	113	35				
12	109	4	63	30				
13	70	16	65	5				
14	80	11	80	5				
15	147	53	100	9				

m = Mean weight of spleen
s = Sample standard deviation of mean.
t = time of sacrifice

Controls: m = 101 ± 16 mgm.

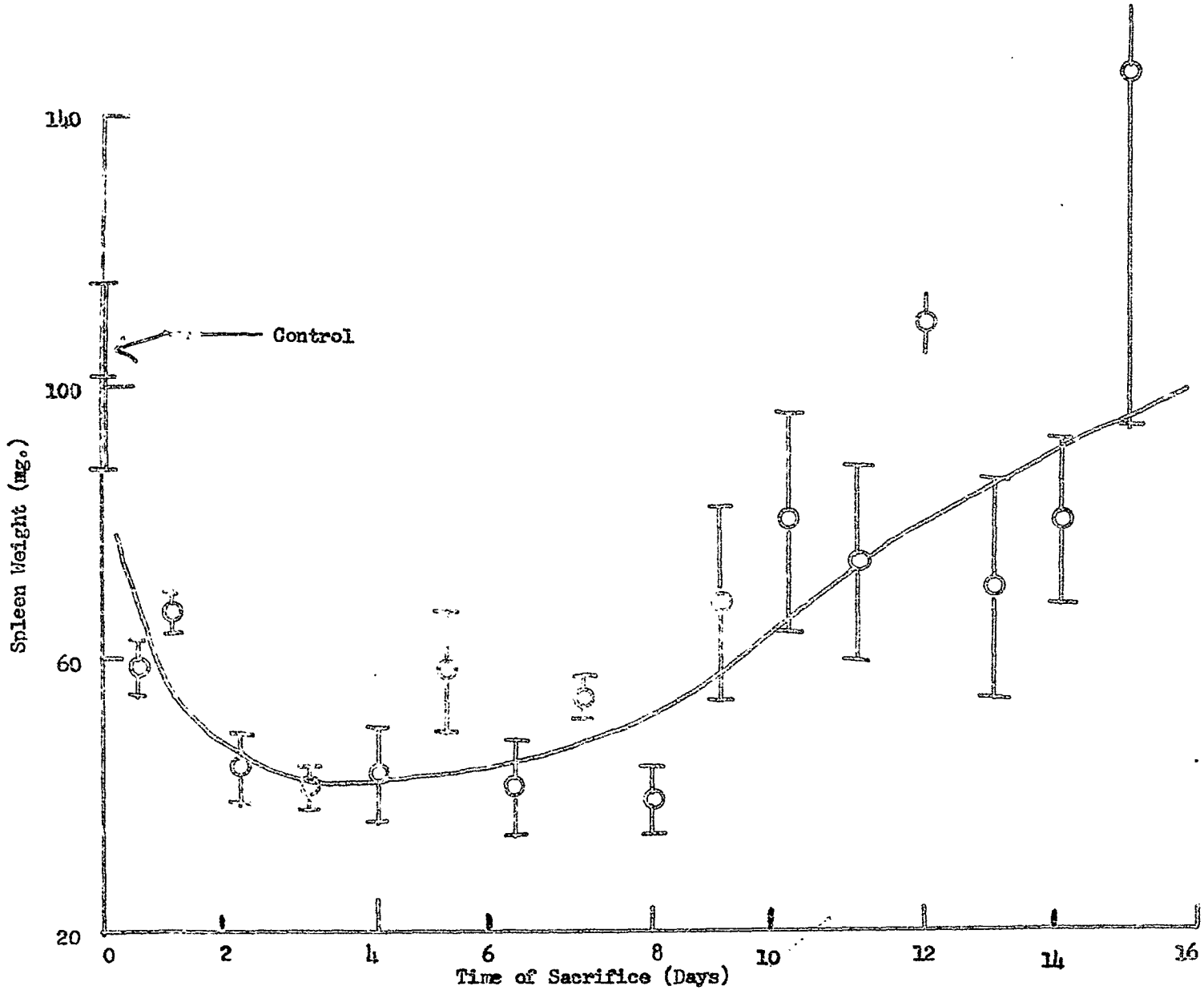

Figure 1. Average spleen weight of mice given vehicle (water) prior to proton irradiation versus time of sacrifice after irradiation.

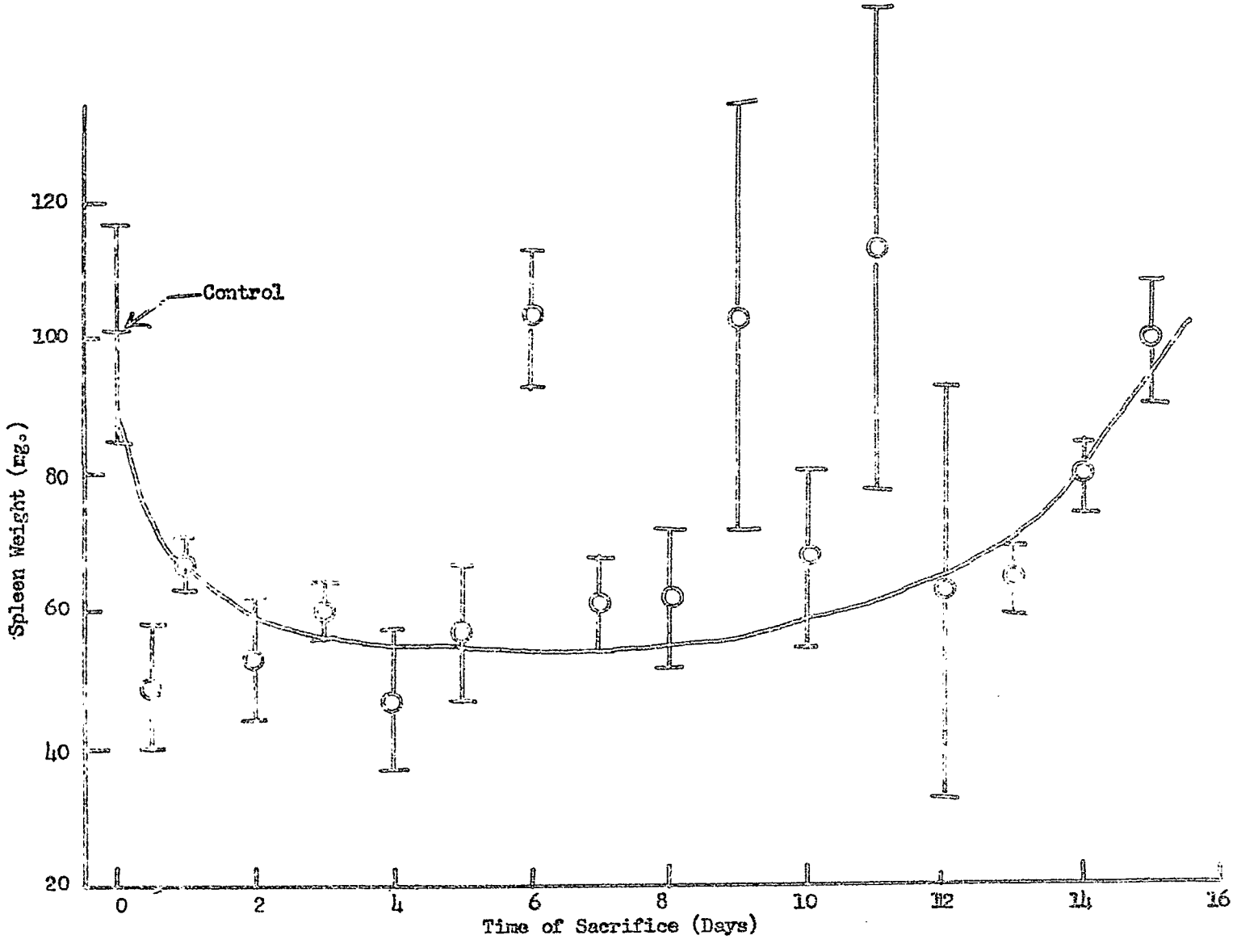

Figure 2. Average spleen weight of mice given MEA prior to proton irradiation versus time of sacrifice after irradiation.

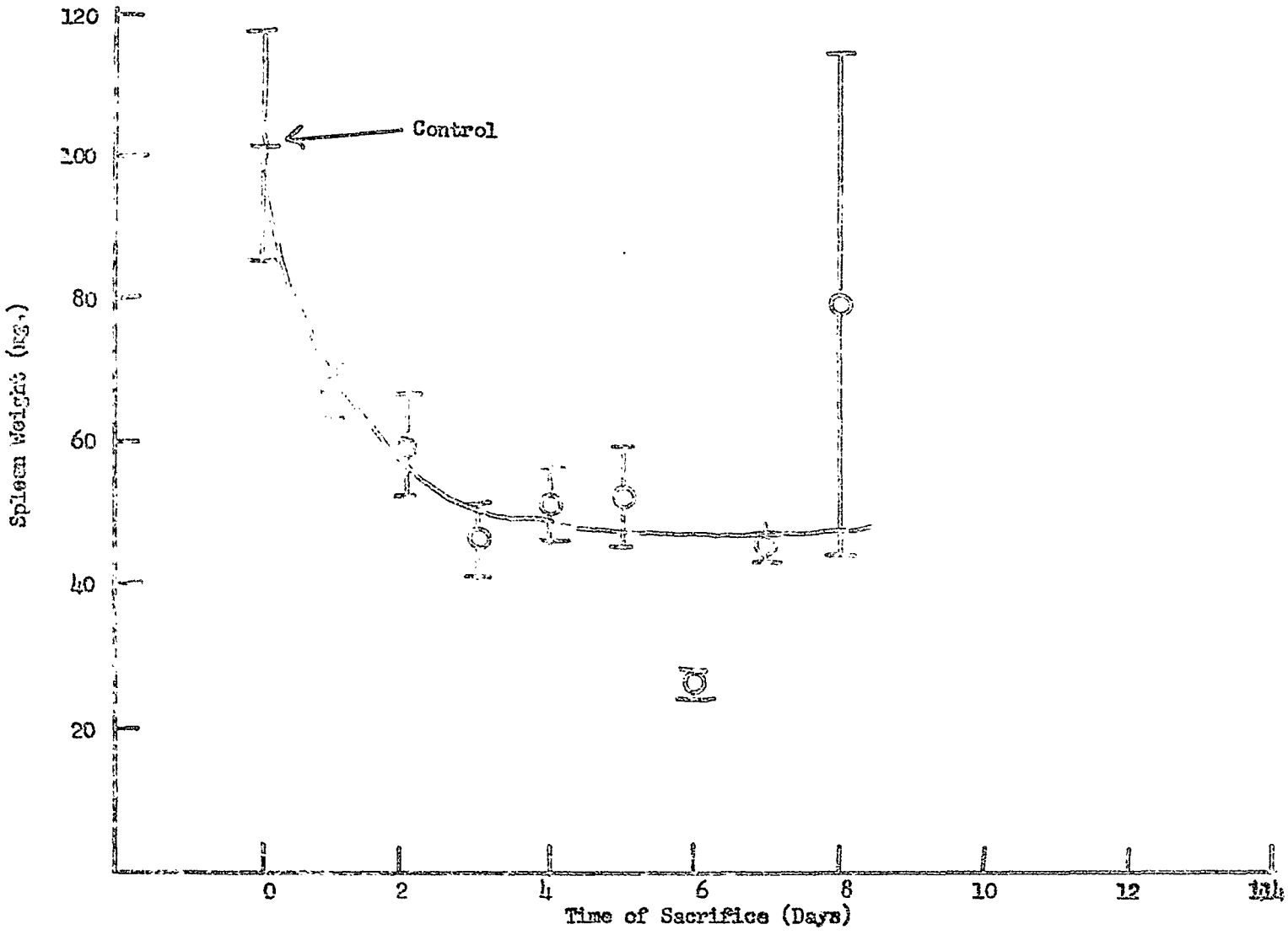

Figure 3. Average spleen weight of mice given vehicle (water) prior to x-ray irradiation versus time of sacrifice after irradiation.

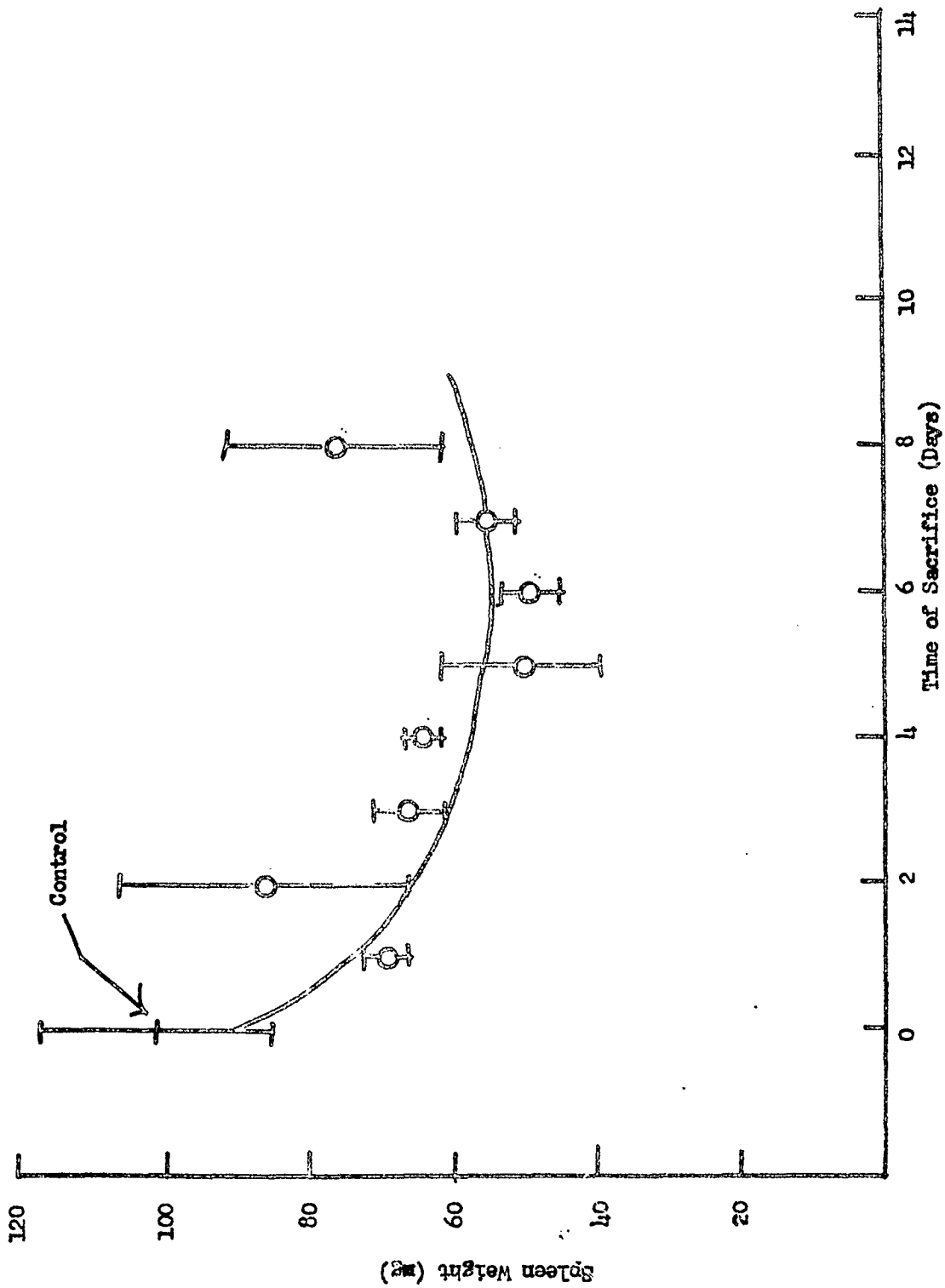

Figure 4. Average spleen weight of mice given MEA prior to x-ray irradiation versus time of sacrifice after irradiation.

TABLE 4

Frequency and Severity of Major Histopathologic Findings in Proton-Irradiated Mice

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice					
		1/2	1	2	3	4	5
Spleen	None						
Atrophy of lymphoid tissue	H_2O	6/6 b_3b_3c c_2c_2c	6/6 c_3c_3c c_3c_3d	6/6 c_2c_3c d_3d_3d	6/6 c_3c_3c d_3d_3d	6/6 b_3c_3c c_3d_3d	6/6 b_3c_3c c_3d_3d
	MEA	3/3 b_3b_3c	3/3 c_2c_3c	3/3 b_3c_3c	3/3 b_3b_3c	3/3 b_3b_3c	3/3 b_3b_3c
Congestion	None						
	H_2O	5/6 a_3a_3a b_3b	6/6 a_3a_3a b_3b_3b	6/6 a_3a_3a a_3b_3c	5/6 a_3a_3a a_3b	6/6 a_3a_3a a_3b_3b	6/6 a_3b_3b b_3b_3c
	MEA	2/3 a_3a	2/3 a_3b	3/3 a_2b_3c	3/3 a_3a_3b	3/3 a_3a_3b	3/3 a_3b_3b
Presence of megakaryocytes	None						
	H_2O	6/6 a_3a_3a a_3b_3b	6/6 a_3a_3a a_3a_3c	6/6 a_3a_3a a_3b_3c	6/6 a_3a_3a b_3b_3b	6/6 a_3a_3b b_3b_3b	6/6 a_3b_3b b_3b_3b
	MEA	3/3 a_3b_3b	3/3 a_3c_3c	3/3 a_3a_3b	3/3 b_3b_3c	3/3 a_3a_3a	3/3 a_3a_3b
Presence of polymorphonuclear leukocytes	None						
	H_2O	6/6 b_3b_3b c_3d_3d	6/6 a_3b_3c c_3c_3c	6/6 a_3a_3a a_2a_3a	4/6 a_3a_3a a	2/6 a_3a	1/6 a
	MEA	3/3 b_3c_3d	3/3 b_3b_3b	3/3 a_3a_3b	2/3 a_3a	0/3	3/3 a_3a_3a

TABLE 4--Continued

(Days Post-irradiation)

6	7	8	9	10	11	12	13	14	15
					0/5				
6/6 c,c,c d,d,d	6/6 c,c,c c,c,c	6/6 c,c,c c,c,d	6/6 b,b,c c,c,d	4/4 b,b,b c	4/4 c,c,c c	4/4 b,b,b b	4/4 b,b,b b	4/4 b,b,b b	3/3 b,b,b
3/3 b,b,b	3/3 b,b,d	3/3 b,b,b	3/3 b,b,c	3/3 b,c,c	3/3 c,d,d	3/3 b,b,b	3/3 c,c,c	2/2 b,c	3/3 b,b,b
					0/5				
6/6 b,b,b b,b,c	6/6 a,a,a b,b,c	6/6 a,a,b b,b,b	6/6 a,a,a a,a,a	3/4 a,a,a	3/4 a,a,a	4/4 a,b,b b	3/4 a,a,a	2/4 a,a	3/3 a,a,a
3/3 b,b,b	3/3 b,b,c	3/3 b,b,b	3/3 b,b,b	3/3 a,b,b	3/3 a,a,a	3/3 a,b,b	3/3 a,b,b	2/2 a,b	3/3 a,b,b
					5/5 b,b,b b,c				
6/6 a,a,a a,b,b	6/6 a,a,a b,b,b	3/6 a,a,a	3/6 a,b,c	4/4 a,a,c d	4/4 a,b,b c	4/4 a,a,b c	4/4 b,b,b b	4/4 b,d,d d	3/3 a,c,c
3/3 b,b,b	3/3 a,a,b	3/3 b,b,d	3/3 c,c,c	3/3 a,b,c	3/3 c,c,d	3/3 b,b,c	3/3 a,b,b	2/2 b,b	3/3 c,d,d
					5/5 a,a,a a,a				
1/6 a	1/6 a	0/6	2/6 a,a	1/4 a	2/4 a,a	3/4 a,a,a	4/4 a,a,a a	3/4 a,a,a	3/3 a,a,a
3/3 a,a,b	1/3 b	3/3 a,b,b	3/3 a,a,b	3/3 a,a,b	3/3 a,b,b	3/3 a,a,a	3/3 a,a,a	2/2 a,a	3/3 a,b,b

TABLE 4--Continued

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice					
		1/2	1	2	3	4	5
Spleen	None						
Recovery of granulocytic elements	H_2O	0/6	2/6 a,a	3/6 a,a,a	4/6 a,a a,a	4/6 a,b b,b	5/6 a,b,b b,c
	MEA	0/3	1/3 b	3/3 a,a,b	3/3 b,a,c	3/3 a,a,b	3/3 b,c,c
	None						
Recovery of erythroblastic elements	H_2O	1/6 a	1/6 a	3/6 a,a,a	2/6 a,a	3/6 b,c,c	5/6 b,c,c c,c
	MEA	0/3	2/3 a,b	3/3 a,a,a	3/3 b,b,b	3/3 a,b,b	3/3 b,b,c
	None						
Hemosiderosis	H_2O	5/6 a,a,a a,a	6/6 a,a,a b,b,b	6/6 a,a,b b,b,b	6/6 a,a,c a,a,b	6/6 a,a,a b,b,b	6/6 a,a,a b,b,b
	MEA	3/3 a,a,a	3/3 a,a,a	3/3 a,a,a	3/3 a,a,b	3/3 a,a,a	3/3 b,b,b
Bone marrow	None						
Hypocellularity	H_2O	4/6 a,a a,b	6/6 a,a,b b,c,c	6/6 a,b,c c,c,c	6/6 c,c,c c,d,d	6/6 b,c,c d,d,d	6/6 b,c,c c,d,d
	MEA	1/3 a	2/3 a,b	2/3 c,c	3/3 b,b,c	2/3 b,d	2/3 a,a

TABLE 4--Continued

(Days Post-irradiation)

6	7	8	9	10	11	12	13	14	15
					2/5 a,a				
2/6 a,a	4/6 a,b,b b	2/6 a,a	4/6 a,a,a a	3/4 a,b,b	3/4 a,a,b	4/4 a,b,c b	4/4 b,b,c c	4/4 b,b,c c	3/3 b,b,c
3/3 c,c,c	2/3 a,c	2/3 b,c	3/3 a,b,c	3/3 a,a,b	3/3 b,b,d	3/3 a,a,c	3/3 a,a,b	2/2 b,c	3/3 d,d,d
					2/5 a,a				
4/6 a,b,b b	5/6 a,b,b c,c	5/6 a,a,a c,c	6/6 a,a,b c,c,d	4/4 c,c,d d	4/4 c,c,c c	4/4 c,c,d d	4/4 c,c,c d	4/4 b,b,c c	3/3 b,b,d
3/3 c,c,d	3/3 a,b,c	3/3 c,c,d	3/3 b,c,c	3/3 b,c,d	3/3 b,c,d	3/3 b,c,d	3/3 a,b,c	2/2 b,b	3/3 a,c,c
					4/5 a,a,a,a				
6/6 b,b,b b,c,c	6/6 a,a,a b,b,b	6/6 b,b,b c,c,c	6/6 a,a,a b,b,b	4/4 a,b,b b	4/4 a,a,b b	3/4 a,a,b	4/4 a,a,a a	4/4 a,a,b b	2/3 a,a
3/3 a,b,b	3/3 a,a,b	3/3 b,b,b	3/3 b,b,b	3/3 a,b,b	3/3 a,b,b	3/3 a,b,b	3/3 a,b,b	2/2 a,a	3/3 a,a,a
					0/5				
6/6 b,c,c d,d,d	6/6 b,b,d d,d,d	6/6 b,c,d d,d,d	5/6 b,c,d d,d	4/4 b,b,c c	4/4 b,c,c d	4/4 b,b,c c	4/4 a,a,b b	3/4 b,b,b	0/2
3/3 a,b,b	3/3 a,a,b	3/3 b,b,b	2/3 b,c	3/3 a,b,c	1/3 c	3/3 a,a,b	2/3 b,c	1/2 a	0/2

TABLE 4--Continued

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice					
		1/2	1	2	3	4	5
Bone marrow	None						
Recovery of myeloblastic and erythroblastic elements	H_2O	0/6	0/6	0/6	0/6	0/6	4/6 b,b c,c
	MEA	0/3	0/3	1/3 b	2/3 b,b	2/3 a,b	2/3 c,c
Congestion	None						
	H_2O	1/6 b	6/6 a,a,a b,b,b	5/6 a,b,b c,c	6/6 b,b,b b,c,c	6/6 a,b,b b,b,b	5/6 a,b,b c,c,d
	MEA	1/3 a	3/3 a,b,b	2/3 b,b	3/3 b,b,b	2/3 a,a	2/3 a,a
Presence of gelatinous marrow	None						
	H_2O	0/6	0/6	2/6 b,c	5/6 a,a,b b,c	3/6 a,a,b	5/6 b,c,c c,d
	MEA	0/3	0/3	0/3	0/3	0/3	0/3
Thymus	None						
Atrophy	H_2O	6/6 b,b,b b,b,c	6/6 b,b,b b,b,c	6/6 a,b,b b,c,c	4/4 b,b,c c	6/6 b,b,b c,c,d	6/6 a,c,c c,c,c
	MEA	3/3 a,b,b	3/3 b,c,c	3/3 a,b,b	3/3 b,c,c	3/3 c,c,c	2/3 a,b

TABLE 4--Continued

(Days Post-irradiation)

6	7	8	9	10	11	12	13	14	15
					0/5				
2/6 a,b	2/6 a,b	3/6 a,a,b	3/6 a,c,d	3/4 a,b,b	2/4 a,c	4/4 b,b,b c	3/4 c,c,d	4/4 b,c,c d	2/2 c,c
3/3 b,c,c	3/3 a,b,c	3/3 b,b,c	3/3 a,c,d	3/3 a,b,c	3/3 a,d,d	3/3 b,c,c	3/3 a,b,d	2/2 c,d	2/2 d,d
					0/5				
6/6 b,b,b b,b,c	6/6 a,c,c c,c,d	6/6 a,a,b b,c,c	6/6 a,a,a b,b,c	4/4 b,b,b a	4/4 a,b,c c	4/4 a,c,c c	3/4 a,b,c	3/4 a,b,b	1/2 b
3/3 a,a,b	3/3 b,b,c	3/3 a,a,b	2/3 a,b	3/3 a,b,b	1/3 b	3/3 a,a,b	2/3 a,b	2/2 a,a	0/2
					0/5				
5/6 a,b,b c,c	6/6 a,b,b c,c,c	6/6 a,a,a b,b,b	2/6 c,c	1/4 a	1/4 b	2/4 a,b	1/4 a	0/4	0/2
0/3	0/3	0/3	0/3	1/3 a	0/3	0/3	0/3	1/2	1/2
					0/5				
6/6 b,b,c c,c,c	5/6 a,a,c c,c	3/4 a,b,b	3/6 a,b,c	0/4	0/4	1/4 b	0/4	0/4	1/3 a
2/3 b,b	0/3	1/3 a	2/3 a,b	1/3 a	0/3	1/3 a	0/3	0/3	1/3 b

TABLE 4--Continued

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice					
		1/2	1	2	3	4	5
Lymph nodes Atrophy	H_2O	6/6 a_5b_5b b_5b_5c	6/6 a_5a_5b b_5b_5b	6/6 a_5a_5b b_5c_5c	4/4 b_5b_5b b	6/6 a_5b_5b b_5c_5b	6/6 a_5b_5b b_5b_5b
	MEA	3/3 a_5b_5b	3/3 a_5a_5b	3/3 a_5b_5b	3/3 b_5b_5c	3/3 a_5b_5c	2/3 a_5a
Testis Focal aspermatogenesis	None						
	H_2O	0/6	2/6 a_5a	4/6 a_5a b_5c	3/6 a_5a_5b	3/6 a_5b_5b	3/6 a_5a_5a
	MEA	3/3 a_5a_5b	3/3 a_5a_5b	2/3 a_5b	1/3 a	2/3 a_5a	2/3 a_5a
Liver Peliosis hepatis-like lesion	None						
	H_2O	0/6	0/6	0/6	0/6	0/6	0/6
	MEA	0/3	0/3	0/3	0/3	0/3	0/3
Irregularity in the size and shape of the hepatic cell nuclei	None						
	H_2O	1/6 a	5/6 a_5b_5b b_5b	6/6 a_5b_5b b_5b_5b	6/6 a_5a_5b b_5b_5b	5/6 a_5a_5b b_5b	6/6 a_5a_5b b_5b_5b
	MEA	3/3 a_5a_5b	1/3 b	3/3 b_5b_5c	2/3 a_5a	2/3 a_5a	1/3 a
Brain Scattered focal hemorrhages	None						
	H_2O	1/6 a	0/6	0/6	3/6 a_5a_5a	0/6	0/6
	MEA	0/3	0/3	1/3 a	1/3 a	0/3	0/3

TABLE 4--Continued

(Days Post-irradiation)

6	7	8	9	10	11	12	13	14	15
6/6 b,b,b b,b,b	5/6 a,a,b b,b	3/4 a,b,b	4/6 a,a,b c	0/4	1/4 a	1/4 b	0/4	0/4	1/3 a
2/3 a,b	0/3	1/3 a	2/3 a,a	0/3	0/3	1/3 a	0/3	0/2	1/3 a
					0/5				
5/6 a,a,b b,d	5/6 a,a,a b,b	5/6 a,a,a b,b	6/6 a,a,a b,b,c	4/4 a,a,a c	4/4 a,a,b d	4/4 a,a,b c	3/4 a,b,c	4/4 a,a,b b	3/3 a,b,c
2/3 a,b	2/3 b,b	2/3 b,b	2/3 a,b	2/3 a,b	2/3 a,b	3/3 a,a,a	3/3 a,b,b	2/3 a,b	2/3 a,b
					0/5				
0/6	0/6	0/6	0/6	0/4	1/4 b	1/4 a	0/4	0/4	1/3 b
0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 b	1/3 a	0/3
					2/5 b,b				
4/6 a,a,b b	6/6 a,b,b b,b,b	6/6 a,b,b b,b,b	6/6 b,b,b b,b,b	2/4 b,c	3/4 a,a,b	3/4 a,a,a	4/4 a,a,b b	2/4 a,a	2/3 a,b
1/3 b	2/3 a,b	2/3 a,b	3/3 a,b,b	3/3 a,b,b	3/3 a,b,b	1/3 b	1/3 a	1/3 b	1/3 a
					0/5				
0/6	0/6	0/6	0/6	0/4	0/4	0/4	0/4	0/4	0/3
0/3	0/3	0/3	0/3	2/3 a,a	0/3	0/3	1/3 b	0/3	0/3

TABLE 5

Frequency and Severity of Major Histopathologic Findings in X-irradiated Mice

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice (days Post-irradiation)							
		1	2	3	4	5	6	7	8
Spleen Atrophy of the lymphoid tissue	H_2O	4/4 $b_3c_3c_3c$	4/4 $b_3b_3c_3c$	3/3 b_3c_3c	4/4 $c_3c_3c_3c$	4/4 $b_3b_3c_3c$	4/4 $b_3c_3c_3d$	4/4 $b_3c_3c_3c$	4/4 $b_3b_3c_3d$
	MEA	3/3 b_3c_3d	4/4 $b_3b_3c_3c$	4/4 $b_3b_3b_3b$	4/4 $b_3b_3b_3c$	4/4 $c_3c_3c_3c$	3/3 c_3c_3d	2/2 b_3c	2/2 b_3b
Congestion	H_2O	4/4 $a_3a_3a_3a$	4/4 $a_3a_3a_3b$	3/3 a_3a_3b	4/4 $a_3a_3a_3b$	4/4 $b_3b_3b_3b$	4/4 $a_3a_3b_3b$	4/4 $a_3a_3b_3b$	4/4 $a_3a_3b_3b$
	MEA	3/3 a_3a_3a	4/4 $a_3a_3a_3a$	3/4 a_3a_3a	4/4 $a_3a_3a_3b$	4/4 $a_3a_3a_3b$	3/3 a_3a_3a	2/2 a_3a	2/2 a_3b
Presence of megakaryocytes	H_2O	4/4 $b_3c_3c_3d$	4/4 $b_3c_3c_3d$	3/3 a_3b_3c	4/4 $a_3a_3a_3b$	3/4 b_3b_3b	3/4 a_3a_3b	4/4 $a_3a_3b_3b$	4/4 $a_3a_3b_3d$
	MEA	3/3 b_3c_3c	4/4 $b_3c_3c_3d$	3/3 b_3c_3d	4/4 $a_3b_3b_3b$	4/4 $a_3a_3b_3b$	3/3 b_3b_3b	2/2 b_3b	2/2 b_3c
Recovery of granulocytic elements	H_2O	1/4 a	2/4 a_3a	3/3 a_3a_3b	4/4 $b_3b_3b_3c$	4/4 $a_3a_3b_3c$	3/4 a_3a_3b	2/4 a_3a	2/4 a_3c
	MEA	3/3 a_3a_3b	4/4 $b_3b_3c_3c$	3/3 b_3c_3c	4/4 $b_3b_3b_3b$	3/4 a_3a_3c	3/3 a_3a_3b	2/2 a_3b	2/2 a_3a

TABLE 5—Continued

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice (Days Post-Irradiation)							
		1	2	3	4	5	6	7	8
Presence of polymorphonuclears	H_2O	4/4 b,b,c,c	4/4 a,a,b,b	3/3 a,a,b	4/4 a,a,a,a	1/6 a	0/4	2/4 a,a	1/4 a
	MEA	3/3 a,a,b	2/4 a,a	1/4 a	2/4 a,a	0/4	0/3	2/2 a,a	1/2 a
Recovery of erythroblastic elements	H_2O	2/4 a,a	2/4 a,a	0/3	4/4 a,b,b,c	3/4 a,b,b	4/4 a,b,b,c	4/4 b,b,b,c	4/4 a,b,b,d
	MEA	0/3	4/4 a,a,b,c	3/3 b,c,c	4/4 a,a,b,b	4/4 a,b,c,d	3/3 b,b,c	2/2 b,b	2/2 b,c
Hemosiderosis	H_2O	4/4 a,a,a,a	4/4 a,a,a,a	3/3 a,a,b	4/4 b,b,b,c	4/4 a,a,b,b	4/4 a,a,b,b	4/4 b,b,b,b	4/4 a,a,b,b
	MEA	3/3 a,a,b	4/4 a,a,a,a	3/3 a,b,b	4/4 a,a,b,b	4/4 a,a,a,a	3/3 a,b,c	3/3 a,b,c	2/2 a,a
Bone Marrow Hypocellularity	H_2O	3/3 a,a,a	3/3 c,d,d	4/4 c,c,c,c	1/1 c	3/3 c,c,c	2/2 b,c	3/4 c,c,c	4/4 b,b,c,d
	MEA	2/3 b,b	4/4 b,b,c,c	4/4 b,b,b,b	4/4 a,b,b,b	4/4 b,b,b,b	3/3 a,a,a	3/3 b,c,c	2/2 b,d
Recovery of granulocytic elements	H_2O	0/3	0/3	0/4	0/1	3/3 a,a,a	1/2 b	2/4 a,a	3/4 b,b,c
	MEA	1/3 a	0/4	3/4 b,b,b	4/4 a,a,b,b	4/4 b,b,c,c	3/3 c,c,c	2/3 b,b	1/2 b

TABLE 5--Continued

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice (Days Post-Irradiation)							
		1	2	3	4	5	6	7	8
Bone marrow Congestion	H_2O	2/3 b,b	2/3 b,c	4/4 c,c,c,c	1/1 b	3/3 b,c,c	2/2 a,b	3/4 a,a,a	4/4 a,b,c,d
	MEA	2/3 b,b	4/4 a,b,b,c	3/4 b,b,c	4/4 b,b,b,c	4/4 a,b,b,c	3/3 a,b,b	3/3 a,b,b	2/2 a,b
Gelatinous marrow	H_2O	0/3	1/3 b	1/4 a	0/1	1/3 a	0/2	1/4 a	0/4
	MEA	0/4	0/4	0/4	0/4	0/4	0/3	0/3	0/2
Thymus Atrophy	H_2O	4/4 b,c,c,b	4/4 a,b,b,b	4/4 c,c,c,c	4/4 b,b,c,c	2/4 a,c	2/4 a,b	2/3 a,b	1/4 b
	MEA	3/3 a,b,b	3/3 b,b,b	3/3 b,c,c	2/3 a,b	2/3 a,b	2/3 b,b	1/3 a	1/3 a
Lymph nodes Atrophy	H_2O	4/4 b,b,c,c	4/4 b,b,b,c	4/4 b,b,b,b	4/4 b,b,b,b	2/4 a,c	2/4 a,b	3/4 a,a,a	2/4 a,b
	MEA	3/3 a,b,b	4/4 a,a,b,b	3/3 b,b,b	2/3 a,b	2/3 a,a	3/3 b,b,b	1/3 a	1/3 a

TABLE 5—Continued

Major Histopathologic Findings	Treatment	Time of Serial Sacrifice (Days Post-Irradiation)							
		1	2	3	4	5	6	7	8
Testis	H_2O	3/4 a,a,a	3/4 a,a,b	4/4 a,a,b,c	4/4 a,a,a,b	3/4 a,b,b	3/4 a,a,b	4/4 a,a,b,c	4/4 a,a,b,b
Focal aspermatogenesis	MEA	3/4 a,a,a	3/4 a,a,a	3/4 a,b,b	2/4 b,b	3/4 a,b,b	3/4 a,b,c	3/3 a,a,b	2/2 a,b
Liver	H_2O	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Peliosis hepatis-like lesions	MEA	0/4	0/4	0/4	0/4	0/4	0/3	1/3 a	1/3 a
Irregularity in size and shape of the hepatic cell nuclei	H_2O	4/4 a,b,b,b	3/4 a,b,b	4/4 b,b,b,b	4/4 b,b,b,c	4/4 b,b,b,b	4/4 b,b,b,b	4/4 a,b,b,b	4/4 a,a,b,b
	MEA	3/3 a,b,b	4/4 a,a,b,b	3/4 a,b,b	3/4 a,b,b	1/6 b	2/3 b,c	2/3 a,b	2/3 a,b
Brain	H_2O	0/4	0/4	0/4	1/4 a	0/4	0/4	0/4	0/4
Scattered focal hemorrhages	MEA	0/4	0/4	0/4	0/4	0/4	0/3	0/3	0/3

Spleen. There was moderate to marked atrophy of the lymphoid tissue in all of the animals in both protected and unprotected groups. The severity of these changes appeared to be less in animals treated with MEA prior to proton exposure. In unprotected animals, the atrophy was most severe in the mice sacrificed from two to six days. Mild to moderate congestion was a frequent finding in most of the mice exposed to protons whether they were treated with MEA or not. Megakaryocytes were present in mild to moderate degree up to the seventh day but were more numerous in mice killed on the eighth day and at later times. Polymorphonuclears were seen in proton-irradiated mice with high frequency up to the second day. Beyond this the frequency of occurrence dropped drastically in unprotected mice but much less drastically in MEA-treated mice.

An interesting finding was the faster recovery of the hematopoietic system of the animals given MEA prior to exposure. In this group, recovery of both granulocytic and erythroblastic elements was observed in mild to moderate degree in almost all mice killed from the second day on. On the other hand, in mice given protons without protection, recovery of hematopoietic cells was only partial during the second week. Extramedullary hematopoiesis was present in the spleen sections of all control mice in mild to marked degree. Hemosiderosis was noted in all of the animals examined in varying degrees.

In the x-irradiated mice there was atrophy of lymphoid tissue which did not appear to be consistently different in mice given MEA prior to x-ray exposure. Faster recovery of hematopoietic cells, particularly of the granulocytic series, was observed in most of the mice sacrificed from the second day on. Both protected and unprotected animals had mild to moderate congestion of the spleen. Megakaryocytes were present in almost all x-rayed animals regardless of the treatment. Polymorphonuclear leukocytes were observed more frequently in unprotected animals during the first six days than in protected mice.

Bone marrow. In most of the mice exposed to the proton radiation, hypocellularity of the bone marrow in moderate to very marked degree was observed. Hypocellularity was less frequent and less severe in mice pretreated with MEA. In bone marrow sections of mice given protons, the only cells which remained were the sinusoid lining-cells, a few macrophages, reticular and fat cells, and some quite abnormal megakaryocytes. Minimal cellular debris was observed in mice killed 12 hours post-irradiation. This general cellular depletion detectable at 3 days persisted in proton-irradiated animals until the eighth day. Depleted marrow was characterized mainly by greatly widened sinuses and by the replacement of most of the hematopoietic marrow cells resulting in a gelatinous marrow. The latter effect was seen predominantly in unprotected mice killed from the second to the eighth days. Congestion of bone marrow was a frequent finding in proton-irradiated mice; pretreatment with MEA decreased the severity of this effect. The x-rayed mice also exhibited hypocellularity and congestion of the bone marrow. Both of these effects were somewhat less marked in the mice treated with MEA. The gelatinous marrow condition observed here was very mild in severity and was never as marked as in the mice given protons. Enhanced recovery in MEA-treated animals was seen. At first only erythroblasts could be seen, while later hematopoiesis was largely granulocytic.

Thymus and lymph nodes. Atrophy of the thymus was a frequent finding in the proton-irradiated mice. Treatment with MEA prior to irradiation prevented these changes only slightly with repopulation of the cortex by lymphocytes

appearing to be somewhat accelerated. Inversion of the normal cortical-medullary relation was observed in some of these animals at the second day post-irradiation. In the thymus a sheet of epithelial and vacuolated stroma cells replaced the normal cortex. Pyknosis and quite heavy debris, especially in the outer cortex, were observed within 12 hours after irradiation.

The histological effects of proton irradiation on mesenteric and mediastinal lymph nodes were similar to those seen in the thymus except that lymphoid atrophy was often accompanied by sinusoidal dilatation. In MEA-treated animals all of these alterations were less severe.

Mild to marked atrophy of the lymphoid tissue of the thymus and of the lymph nodes was observed in the mice exposed to x-rays and these changes were diminished in MEA-pretreated animals.

Testis. There was mild to marked atrophy of seminiferous tubules in the mice exposed to proton radiation. This change was slightly milder in mice treated with MEA prior to exposure. The microscopic picture was characterized by a marked decrease in the number of germinal cells, particularly spermatogonia. Appearance of vacuolated and other bizarre spermatogonia and spermatocytes, together with a decreased number of sperm were seen. Mitotic figures were absent in the cells of some tubules and markedly decreased in many others. In most of the sections from these animals, a mixture of atrophic and active seminiferous tubules were seen.

Focal aspermatogenesis mostly mild in degree was observed in the testis in most of the x-irradiated mice with or without MEA treatment.

Liver. In a few animals given protons, both with and without MEA treatment, hemorrhagic lesions reminiscent of peliosis hepatis were observed. They were scattered throughout the liver section and were moderate in degree. The histopathological picture was characterized by dissolution of the liver framework and extravasation of blood into the spaces so formed.

Another finding, more pronounced in x-rayed than in proton-irradiated mice, was irregularities in the size and shape of the hepatic cell nuclei. These changes were less severe in the animals treated with MEA prior to exposure.

Focal hemorrhages similar to those seen in some of the proton-irradiated mice were observed in liver sections from two MEA-treated mice killed on the seventh and eighth days after x-irradiation. The irregularity in size and shape of nuclei was present in moderate degree in x-irradiated animals and was slightly less frequent in MEA-pretreated mice. Moderate to marked focal necrosis of the liver was observed in several of the x-rayed mice.

Brain. Focal hemorrhages were observed in the cerebral and cerebellar gray matter in some of the proton-irradiated animals. Hemorrhages were in some cases confined to the subdural space only. Only one of the x-irradiated animals had a hemorrhage and this was of the subdural type.

Lung. A mild to moderate degree of peribronchial and perivascular accumulation of chronic inflammatory cells was present in most of the control and irradiated animals. Acute and chronic bronchitis and pneumonitis was observed in

a few mice given proton radiation both with and without MEA pretreatment. Mild to moderate congestion was present in a few experimental animals. Atelectasis, emphysema, and hemosiderosis were observed in a few animals again unrelated to treatment.

Perivascular accumulation of chronic inflammatory cells, acute bronchitis, and pneumonitis were observed in x-rayed mice with or without treatment with MEA. Mild congestion was present in lung sections of a few x-rayed mice.

Kidney. Perivascular accumulation of chronic inflammatory cells was seen in mild to moderate degree in some of the control and irradiated animals. Congestion was found in sections from a few animals and in one mouse a moderate degree of hemorrhage was observed. In other animals given protons, cystic dilatation of the pelvis was seen. Similarly, in the mice given x-rays, congestion and perivascular accumulation of chronic inflammatory cells were observed, both in mild degree.

Discussion

The gross and microscopic observations presented in this report are results of our continuing effort to compare and evaluate differences in the biological effects of high-energy protons and x-irradiation and modification of their effects by chemical protective agents. It is apparent from these studies and those in our previous report (2) that acute proton exposure is associated with a decrease in spleen weight; atrophy of lymphoid tissue in the spleen, thymus, and lymph nodes; hypocellularity of the bone marrow; atrophy of the testis; peliosis hepatis-like lesions in the liver; and hemorrhages in the brain. All of these effects were also seen in x-irradiated mice. Thus, it would appear that differences in the pathology from these two types of radiation are more quantitative than qualitative.

However, when MEA was given prior to radiation, differences which may be regarded as qualitative did appear. Thus, the atrophy of the spleen was decreased in mice treated with MEA prior to proton exposure. This protective effect was not observed in the spleens of x-irradiated mice. A second interesting finding was a differentially enhanced hematopoietic recovery in the mice treated with MEA prior to exposure. MEA stimulated recovery of both the granulocytic and erythroblastic elements, but it appeared, although the differences were slight, that granulocytic recovery was more pronounced in proton-irradiated mice during the first few days after irradiation, while in the x-irradiated mice erythroblastic elements predominated over granulocytic. This difference was observed only at 3, 4, and 5 days and disappeared later.

A number of other findings, not as unexpected as the preceding ones, but still having interesting implications were made. Hypocellularity of bone marrow was decreased in the animals that had MEA pretreatment, and enhanced cellular recovery was observed. In the bone marrow sections, early recovery proceeded mainly via myeloblastic elements regardless of the kind of radiation.

Another interesting pathologic finding in the present study was atrophy of seminiferous tubules in the testes of irradiated mice. This lesion was slightly more pronounced in proton-irradiated mice and was less severe in

MEA-treated animals. There was only a slight reduction of spermatogonia and spermatocytes in both proton and x-rayed animals. Loss of spermatogenesis was observed in proton-irradiated mice by the eleventh post-irradiation day.

Kaplan and Lyon (3) and Maisin et al. (4,5) have indicated that MEA does not protect germ cells against x-ray injury. On the other hand, other investigators (6,7) have suggested that MEA can inhibit the transient, sterilizing effect of ionizing radiation.

The third interesting finding was hemorrhage in the liver and brain. The hemorrhagic lesions in the liver were quite reminiscent of peliosis hepatis in humans. Inflammatory changes, liver cell necrosis, and a variety of other factors have been implicated in the pathogenesis of peliosis hepatis in humans (8-11). Kent and Thompson (12) suggested that the development of the blood pools is produced by dilatation of certain groups of sinusoids which subsequently fill with blood. Such liver lesions were observed here in one x-rayed mouse.

Summary

1. The radiation-induced decrease and subsequent recovery of spleen weight is similar in proton- and x-irradiated mice; some protection against splenic weight loss by MEA was observable for both types of radiation.

2. Atrophy of the lymphoid tissue in the spleen, thymus, and lymph nodes was observed in mice exposed to 440 Mev proton or 250 Kvp x-ray. In proton-irradiated mice, MEA decreased the severity of this effect in the spleen while in x-irradiated mice MEA did not produce this effect.

3. Enhanced recovery of hematopoietic cells was observed in MEA-treated mice, granulocytic elements being first to recover in proton-irradiated mice while erythroblastic elements were the first to appear in x-rayed mice.

4. Bone marrow hypocellularity, as well as gelatinous marrow, was less frequently observed in MEA-treated animals.

5. Enhanced hematopoietic recovery of the bone marrow was predominantly myeloblastic regardless of the type of radiation used.

6. Focal aspermatogenesis produced by proton and x-irradiation appeared to be diminished by pretreatment with MEA.

7. Lesions similar to peliosis hepatis in humans were observed in proton-irradiated mice with or without MEA treatment. Similar lesions were observed in one of the x-rayed mice.

8. Subdural hemorrhages and hemorrhages of the gray matter in cerebrum and cerebellum were present in a few mice exposed to high-energy protons, and in a x-rayed mouse.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA OR FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

IV. The Time-Course of Survival in Proton- or X-irradiated Mice Pretreated with Chemical Protectors

D. G. Oldfield, J. Doull, V. Plzak, A. Hasegawa and A. Sandberg

This report concerns: The time-course of survival in mice total-body irradiated by high-energy (440 Mev) protons or by medium energy (250 Kvp) x-rays with and without pre-irradiation treatment with 2-mercaptoethylamine hydrochloride (MEA) or p-aminopropiophenone (PAPP).

Immediate or ultimate application of the results: The results reported here are required for determining the relations between the survival of proton-irradiated mice with and without chemical preprotection and that of x-irradiated mice similarly treated. The analysis of survival-versus-time functions has relation to histological data already obtained in this laboratory (1,2) detailing the nature, extent, and temporal development of changes following irradiation by high-energy protons and medium-energy x-rays. The analysis is also related to certain methodological questions concerning the quantitative assessment of radiation damage and of protection in experimental animals, and concerning the inter-comparison of such measurements with each other for various doses and types of radiation and chemical protectors.

* * * * * * * *

The present status of the proton protection program at this laboratory may be briefly summarized as follows. The two experimental runs (Series A and Series B) made thus far using 440 Mev protons have shown (3,4,5) that both MEA and PAPP can protect CF_1 male mice in the age range 19 ± 5 weeks against lethality as measured by conventional indices such as $LD_{50/30}$'s and DRF's. But these studies have also shown the existence of a differential protective effect in the sense that, whereas MEA is more effective than PAPP for proton doses in the vicinity of the 30-day midlethal dose for x-ray midlethal doses, the reverse is true, with PAPP being more effective than MEA. This result might be attributed primarily to events occurring during irradiation, and thus relate either to qualitative differences in the nature of the energy deposition produced by protons and x-rays, or to qualitative differences in the interaction of the protectors with chemical species produced during irradiation. Or the result might be attributed primarily to events occurring after irradiation, and thus relate to qualitatively different sequelae which develop from what are initially only quantitative differences produced by irradiation in the presence of the protective agent.

To explore these several possibilities in depth will undoubtedly require some extension of the investigative program in the direction of (a) more accurate determinations of the linear energy transfer distributions for each of the

radiations; (b) comparative measurements of the interaction of protective substances with molecular components of irradiated cells, (c) quantitative histopathologic data on the kinetics of radiosensitive cells in irradiated mice, and (d) analysis of the development of lethality with time in populations of irradiated mice. The present report is concerned with certain aspects of the last problem.

Materials and Methods. Since detailed presentations of the physical and biological techniques employed have been made in previous reports (3,4), only the major features of the experimental design are given here. These are shown in Tables 1 and 2.

Results

In Tables 3 and 4 are presented fractional survival data to 20 weeks post-irradiation obtained in Series A for chemically protected, proton-irradiated mice. Survival data for unprotected mice of Series A which received protons have been given in a prior report (4). Tables 5 and 6 give survival data for the Series B protected and unprotected proton-irradiated groups to 14-15 weeks post-irradiation. Tables 7, 8, and 9 give similar data for the Series A x-ray irradiations to 20 weeks post-irradiation.

The standard deviation in the tables is calculated on the assumption that deaths within each group of mice at a particular dose level obey binomial statistics, and that the fractional survival observed in this group is a reasonable estimate of the fractional survival that would be observed in a large population of mice. The number n is the initial number of mice, not including any that died during irradiation or during the first two days post-irradiation. In the control groups, the number of mice excluded from analysis due to immediate death (during irradiation) or due to short-term death (two days post-irradiation) is negligible. In groups receiving MEA or PAPP before irradiation, the excluded mice comprise about 5 to 15% of the initial number.

In view of the probable multiplicity of processes which can lead to an animal's death, and in view of the variable time intervals that such processes may require in different mice, it is not unreasonable to regard the initial development of mortality in irradiated mice as a random process, formally analogous to radioactive decay. In this case, the fractional survival should decrease exponentially with time. When the data of Tables 3 through 9 are plotted semilogarithmically, the survival points for early deaths do, in fact, follow a straight line reasonably well. The experimental data depart, however, from the simple decay model in two ways: first, there is frequently a lag or delay before any deaths at all occur; second, the "decay" rate is not constant at later times. The behavior of the survival points with time can be better approximated by a set of line segments, fitted by eye to the data, using for each line segment those successive points that lie within the calculated standard deviation. The fitting procedure starts with the initial points and moves progressively to points measured at later times.

All data of Tables 3 through 9 have been plotted and examined, but only selected curves are exhibited for the purpose of discussion at this time. The numerical analysis of all of the data, to be presented in a later report, will be accompanied either by line segments or continuous curves approximating the survival points. In the present report only the major features of the data

TABLE 1

Specification of Irradiation Parameters

Parameter	Protons	X-rays
Accelerator	170-inch synchrocyclotron	General Electric Maximar III or Keleket
Energy	440 Mev	250 Kvp
Flux rate or current	$0.4 - 1.0 \times 10^{10}$ protons/sec.	15 ma
Pulse width	400 microsecs.	8.75 millisec.
Pulse repetition rate	70 pulses/sec.	~~120~~ 60 pulses/sec.
External filter	1.4 gm./cm^2 (Mylar plus air)	1/4 mm. copper plus 1 mm. alum.
Size of external collimator	4 inches x 4 inches	(none)
FSD	(focused beam)	75 cm.
Backscatter material	1/4 inch lucite	1 inch lucite
Beam size at irradiation surface	9 inches x 9 inches	30 or 45 cm.
Approx. dose rate in phantom	40 - 80 rad/min.	40 rad/min

TABLE 2

Specification of Chemical and Biological Parameters

Parameter	Specification
Drug doses	MEA: 225 mgm./kgm.; aqueous solution
	PAPP: 30 mgm./kgm. in 50% aqueous propylene glycol
	Water or propylene glycol: 1% of body weight
Mouse strain	Carworth Farms CF_1
Sex	Male
Age of irradiation	14 to 24 weeks
Caging	12 or fewer mice/7x9x13 stainless cage
Feeding	Rockland Mouse Diet plus water, available *ad libitum*
Survival check	Daily to 30 days; weekly thereafter

TABLE 3

Fractional survival versus Time Post-Irradiation for Mice Given PAPP Prior to Proton Irradiation (Series A)

D (rads) / t (Days)	235	351	468	591	702	820
3	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .02	1.00 ± .02
6					.97 .03	
9						.94 .04
12				.94 .04	.88 .06	.91 .05
15					.82 .07	.88 .06
18				.89 .05		.85 .06
21		.97 .03		.86 .06		
28						
37			.94 .04			
42			.80 .07			.82 .07
49			.74 .07	.80 .07	.79 .07	.79 .07
56				.77 .07		
63						
70	.97 .03					
77						
84		.95 .04				
91						
100		.92 .04				
105						
114						
121						.77 .07
126	.95 .04	.87 .06	.71 .08	.74 .07		
133		.84 .06	.69 .08		.77 .07	
140						
m	36	37	35	35	34	34

D = absorbed dose; t = post-irradiation time.

m = initial number of mice

Error term is the calculated standard deviation.

TABLE 4

Fractional Survival versus Time Post-Irradiation for Mice Given MEA Prior to Proton Irradiation (Series A)

D (rads) / t (Days)	235	351	468	591	702	820
3	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01
6						
9					.97 .03	
12				.97 .03		.97 .03
15					.95 .04	.86 .06
18						
21				.91 .05		
28	.98 .02			.89 .05	.87 .06	.83 .06
37	.95 .03				.81 .06	
42					.78 .07	.74 .07
49						.69 .08
56						.66 .08
63						
70		.97 .03			.76 .07	
77	.93 .04		.97 .03	.86 .06		.63 .08
84			.95 .04	.83 .06		
91			.92 .04			
100						
105	.90 .05				.73 .07	.60 .08
114			.87 .06			
121			.84 .06			
126			.81 .06			
133		.95 .04	.78 .07		.70 .08	
140						
m	41	37	37	35	37	35

D = absorbed dose; t = post-irradiation time.

m = initial number of mice.

Error term is the calculated standard deviation.

TABLE 5

Fractional Survival versus Time Post-Irradiation for Mice Given Vehicle Only Prior to Proton Irradiation (Series B)

D (rads) / t (Days)	390[a]	487[a]	618	716	824	935	1037
3	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01
6				.92 .05	.94 .04	.97 .03	.86 .06
9		.97 .03	.83 .06	.78 .07	.86 .06	.78 .07	.56 .08
12	.97 .03		.80 .07	.67 .08	.71 .08	.64 .08	.44 .08
15		.86 .06	.77 .07	.64 .08	.54 .08	.56 .08	.39 .08
18		.81 .07		.61 .08			.36 .08
21					.51 .08	.53 .08	
30			.71 .08	.58 .08	.49 .08		
36-37	.94 .04	.72 .08				.44 .08	
41-42							
48-49							
56-57				.56 .08	.46 .08	.42 .08	.33 .08
62-63							.31 .08
69-70				.53 .08			
76-77							
83-84					.43 .08		
90-91							
97-98	.92 .05	.70 .08		.47 .08			
104-105							
m	36	36	35	36	35	36	36

D = absorbed dose; t = post-irradiation time.

m = initial number of mice

Error term is the calculated standard deviation

[a] Use larger value of t.

TABLE 6

Fractional Survival versus Time Post-Irradiation for Mice Given PAPP or MEA Prior to Proton Irradiation (Series B)

D (rads) / t (Days)	PAPP						MEA					
	873[a]		1116[a]		1347		971[a]		1255		1395	
3	1.00	± .02	1.00	± .01	1.00	± .02	1.00	± .02	1.00	± .02	1.00	± .02
6			.94	.04	.83	.06			.93	.05	.88	.07
9	.97	.03	.89	.05	.62	.08	.97	.03	.72	.08	.63	.10
12	.77	.08	.69	.08			.91	.05	.66	.09	.54	.10
15			.66	.08	.59	.08	.88	.06	.59	.09		
18	.73	.08	.63	.08			.85	.06				
21							.82	.07				
30					.56	.09			.55	.09	.50	.10
37-38												
44-45	.67	.09	.60	.08	.53	.09	.79	.07				
52-53			.57	.08	.47	.09	.67	.08	.48	.09		
58-59					.41	.08	.64	.08	.41	.09		
65-66												
72-73	.63	.09										
79-80												
86-87					.38	.08						
93-94	.60	.09	.54	.08							.46	.10
100-101							.61	.09			.42	.10
m	30		35		34		33		29		24	

D = absorbed dose; t = post-irradiation time.

m = initial number of mice

Error term is the calculated standard deviation.

[a]Use larger value of t.

TABLE 7

Fractional Survival versus Time Post-Irradiation for Mice Given Vehicle Only Prior to X-Irradiation (Series A)

D (rads) / t (Days)	222		333		444		555		666	
3	1.00	± .01	1.00	± .01	1.00	± .01	1.00	± .01	1.00	± .01
6			.97	.03						
9					.97	.03	.92	.05	.86	.06
12			.91	.05	.95	.04	.78	.07	.53	.08
15			.89	.05	.86	.06	.64	.08	.39	.08
18							.53	.08	.36	.08
21	.97	.03			.81	.07	.50	.08		
26			.86	.06	.72	.08	.47	.08		
34					.70	.08	.33	.08	.33	.08
41			.83	.06			.31	.08	.31	.08
48							.25	.07	.29	.08
55										
62										
69					.67	.08				
76										
83										
92	.92	.05	.77	.07						
97	.89	.05	.74	.07	.64	.08				
106			.71	.08			.22	.07		
113	.86	.06	.69	.08						
118	.83	.06			.61	.08				
125			.66	.08						
132	.81	.07	.63	.08	.58	.08				
139					.56	.08			.25	.07
m	36		35		36		36		36	

D = absorbed dose, t = post irradiation time.

m = initial number of mice.

Error term is the calculated standard deviation.

TABLE 8

Fractional Survival versus Time Post-Irradiation for Mice Given PAPP Prior to X-Irradiation (Series A)

D (rads) / t (Days)	444	555	666	777	888
3	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01
6			.97 .03	.97 .03	
9				.94 .04	.94 .04
12	.97 .03		.86 .06	.86 .06	.89 .05
15		.88 .05	.81 .07	.80 .07	.86 .06
18	.90 .05		.78 .07	.77 .07	
21				.71 .08	.83 .06
26	.87 .06		.75 .07	.69 .08	.78 .07
34	.84 .06			.63 .08	.72 .08
41		.85 .06	.67 .08	.57 .08	
48	.82 .06				.67 .08
55					
62	.79 .07				
69	.76 .07				.64 .08
76	.74 .07	.83 .06	.64 .08	.54 .08	
83					
92					
97					
106					.61 .08
113	.71 .07				
118					
125					
132					
139	.63 .03				
m	38	40	36	35	36

D = absorbed dose; t = tpost-irradiation time.

m = initial number of mice.

Error term is the calculated standard deviation.

TABLE 9

Fractional Survival versus Time Post-Irradiation for Mice Given MEA prior to X-Irradiation (Series A)

D (rads) / t (Days)	444	555	666	777	888
3	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01	1.00 ± .01
6					.98 .02
9				.93 .04	.85 .06
12			.95 .04	.76 .07	.61 .08
15			.84 .06	.71 .07	.56 .08
18		.98 .05		.63 .08	.51 .08
21			.82 .06		
26		.95 .03	.74 .07		
34	.95 .03		.71 .07	.61 .08	.49 .08
41				.54 .08	
48				.51 .08	.44 .08
55					.42 .08
62	.93 .04				
69				.49 .08	
76			.68 .08		
83	.90 .05		.66 .08		
92			.63 .08		.34 .07
97		.94 .04			
106			.61 .08	.39 .08	
113	.88 .05			.37 .08	
118		.90 .05			
125	.83 .06				
132					
139	.81 .06		.58 .08	.29 .07	.32 .07
m	41	40	38	41	41

D = absorbed dose; t = post-irradiation time.

m = initial number of mice.

Error term is the calculated standard deviation.

obtained thus far will be described. These can be verified by inspection of the tabular data or of the illustrative curves discussed later.

Speaking generally, the curves for proton-irradiated groups and x-irradiated groups (with or without pretreatment with either protector) are similar. The behavior of all of the curves can be described as were the untreated proton-irradiated groups in a preceding report (4). The initial death rates increase with increasing radiation dose; the initial rate of death is succeeded by a secondary rate of death proportional to, but smaller than, the initial rate; the transition from initial rate to secondary rate occurs earlier in time and at a lower survival for larger doses; for low doses, the initial rate appears to be preceded and followed by periods during which the rate of death is zero.

Discussion

The initial death rates for the various groups can each be approximated by single numbers giving the slope of the line segment through the survival points. Therefore this death process--initial death neglecting any initial lag during which the death rate is zero--has the same functional form (exponentially decreasing with time) for all groups. In this sense, initial death varies only quantitatively over all of the groups. However, when the secondary death rates also are considered and, even more, when the various delays or lags are considered, it is evident that the form of the function which can represent this behavior must be more complicated than exponential to fit the observed survival, even for a single type of radiation and a single protector. It would be permissible to introduce two additional constants: one associated with the particular drug used, its mode of administration, etc.; the other stemming from the fact that the exponential "decay" of survival is not constant. (The values of these constants could be determined from the survival data for various radiation doses with and without protection.) If the data could be fitted by such a procedure, the behavior of the curves for a single type of radiation and a single protector could again be regarded as differing only quantitatively for these groups. If the same function could be used to represent the survival data for both types of radiation and both protectors, the survival behavior could be regarded as qualitatively the same for all groups.

An example of a trial function which might be investigated in this connection is

$$q = e^{-\frac{\alpha D(1 - e^{-\gamma t})}{1 + \beta C}}$$

where q is the fractional number of mice surviving to time t, D is the absorbed dose, and C is the concentration of protector in the animal. The constant α depends on the type of radiation; the constant β, on the type of drug; and the constant γ, on the population of mice used.

However, if, as is likely, the data for both radiations and both protectors could not be fitted by a single function, a much more complicated

situation ensues. The relation one seeks now is not that between a certain set of constants, such as α, β, γ in the equation above, but rather a relation between two or more different survival functions. This relation, when applied to one survival function, must generate the other survival function not merely at one time for one dose but over the entire range of interest of these variables. In other words, we describe the differences in the behavior of two survival functions by specifying the transformations required to convert one into the other. This relation of survival functions to each other constitutes the conceptual mechanism for making comparisons between different regimes of treatment. To pass from the concept to the practical analytical tool requires that the change in type of radiation or type of drug be specified in a quantitative way. For radiation, this specification might be the average linear energy transfer to molecular constituents of a cell; for a drug, it might be some index of reactivity or of extent of reaction with molecular constituents of a cell. The rationale for extending the investigative program can thus be seen to have an analytical basis.

This broad treatment of radiation survival and protection contrasts naturally to the more usual methods of analyzing survival data by restricting the range of the variables. Selecting particular values of the variables as fiducial (e.g., 30-day assay, 50% survival, etc.) yield various familiar indices (e.g., $LD_{50/30}$, ST_{50}, etc.). The gain in simplicity, however, is accompanied by a loss of information regarding the relation of the variables to each other except at the fiducial points. The loss may or may not be significant for any given investigation. In the case of radiation-induced lethality and protection against lethality, where the biological events observed (animal death) is removed in time from the inducing physical event, neglect of time as a variable of interest could easily produce data which was misleading from an interpretative point of view.

An additional point regarding the analysis of the survival data concerns the reproducibility of the survival curves from run to run. The indications from a comparison of Series A with Series B curves for proton-irradiated animals are that (a) reasonable agreement (within 1 standard derivation over the entire curve) occurs in roughly one-quarter of the groups (e.g., Figures 1 and 2); (b) deviations appreciably greater than 1 standard deviation also occur in about one-quarter of the groups (e.g., Figures 3 and 4; (c) about half of the groups lie between these extremes, with agreement between some portions of the curve but not between others (Figures 5 and 6).

The importance of time as a parameter together with the fact that systematic differences between survival curves tend to be obscured by fluctuations in the time-course of survival make it reasonable to commence the detailed analysis of radioprotection using the concept of survivance. This quantity, defined as the time integral of the survival curves between specified limits, has been discussed in a preceding report (4). The use of this quantity can be regarded as a compromise between preserving time as an explicit, untransformed variable and smoothing certain of the fluctuations in survival which occur from experiment to experiment.

Summary

1 Survival versus time in CF_1 male mice total-body irradiated by high-energy (340 Mev) protons or by medium energy (250 Kvp) x-rays with and without

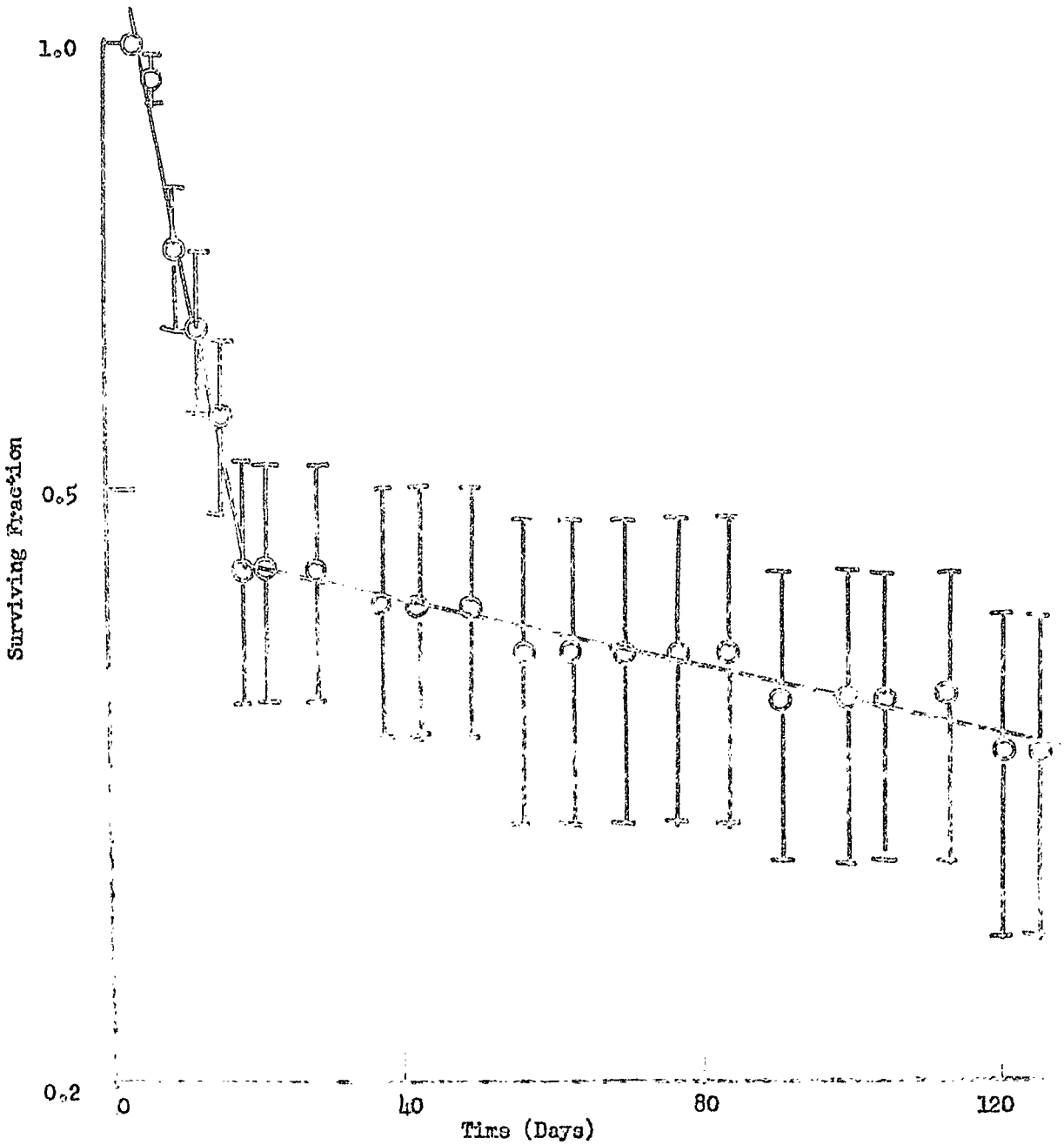

Figure 1. Surviving fraction of mice given vehicle only versus time after 820 rads proton irradiation (Series A).

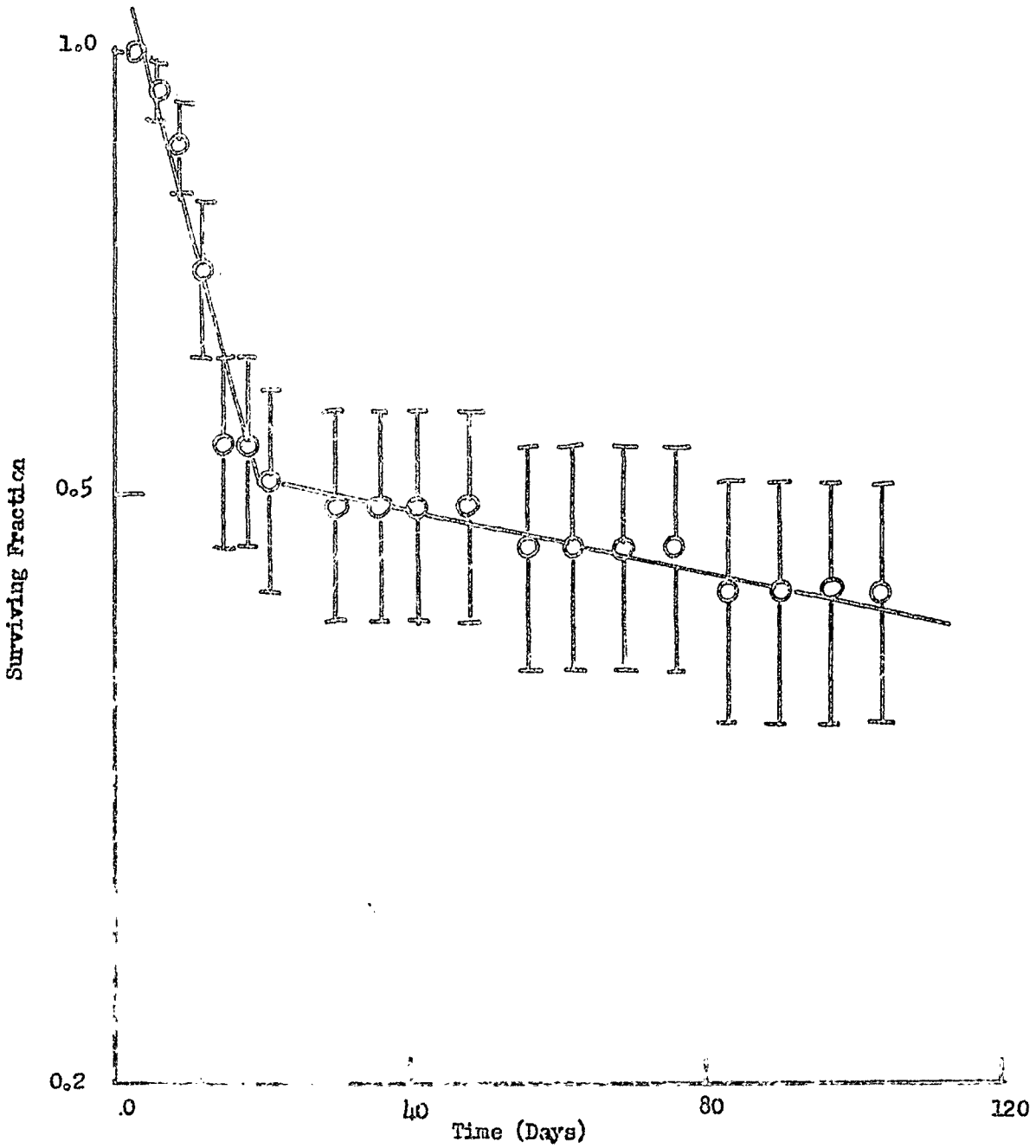

Figure 2. Surviving fraction of mice given vehicle only versus time after 824 rads proton irradiation (Series B).

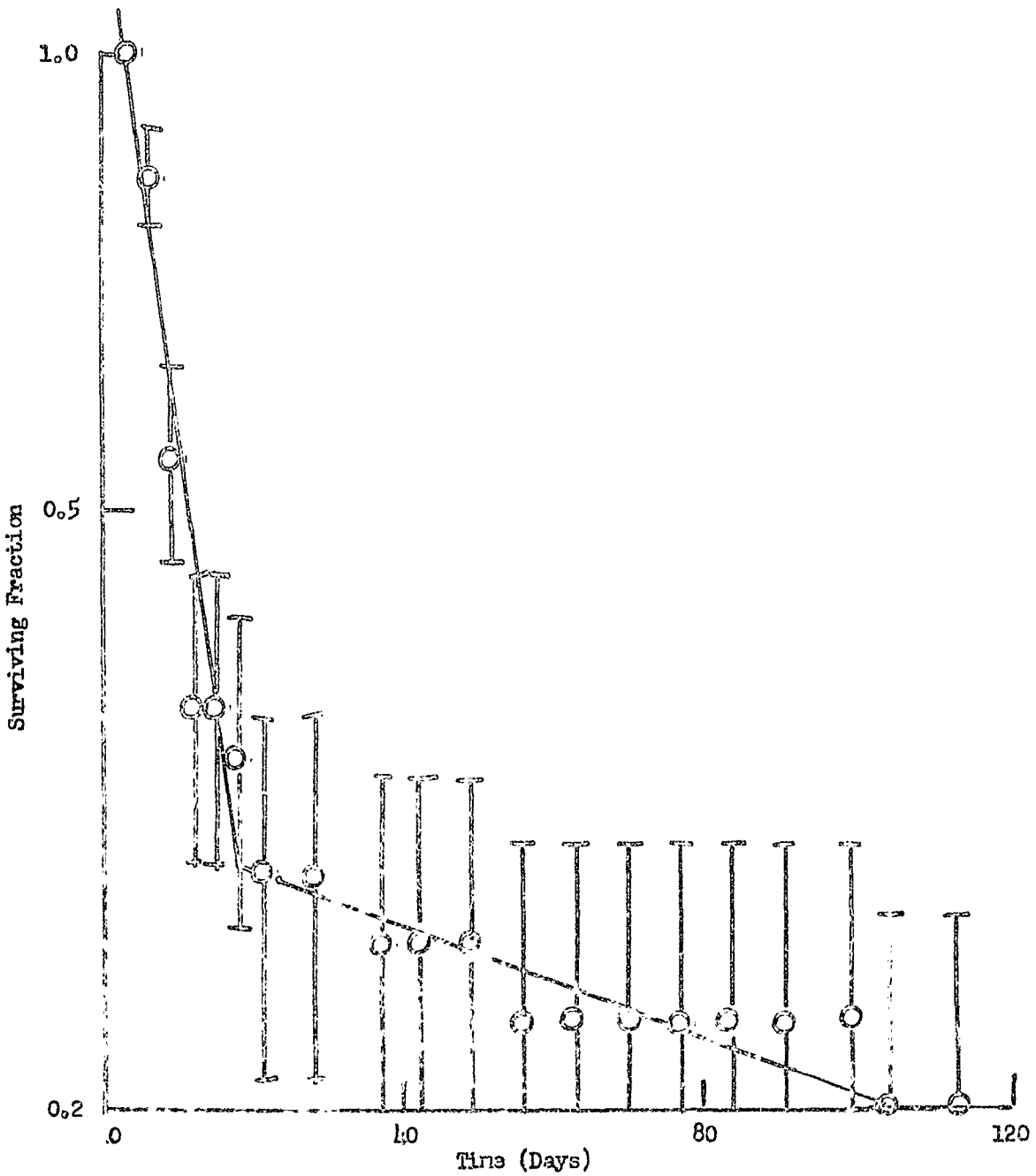

Figure 3. Surviving fraction of mice given vehicle only versus time after 939 rads proton irradiation (Series A)

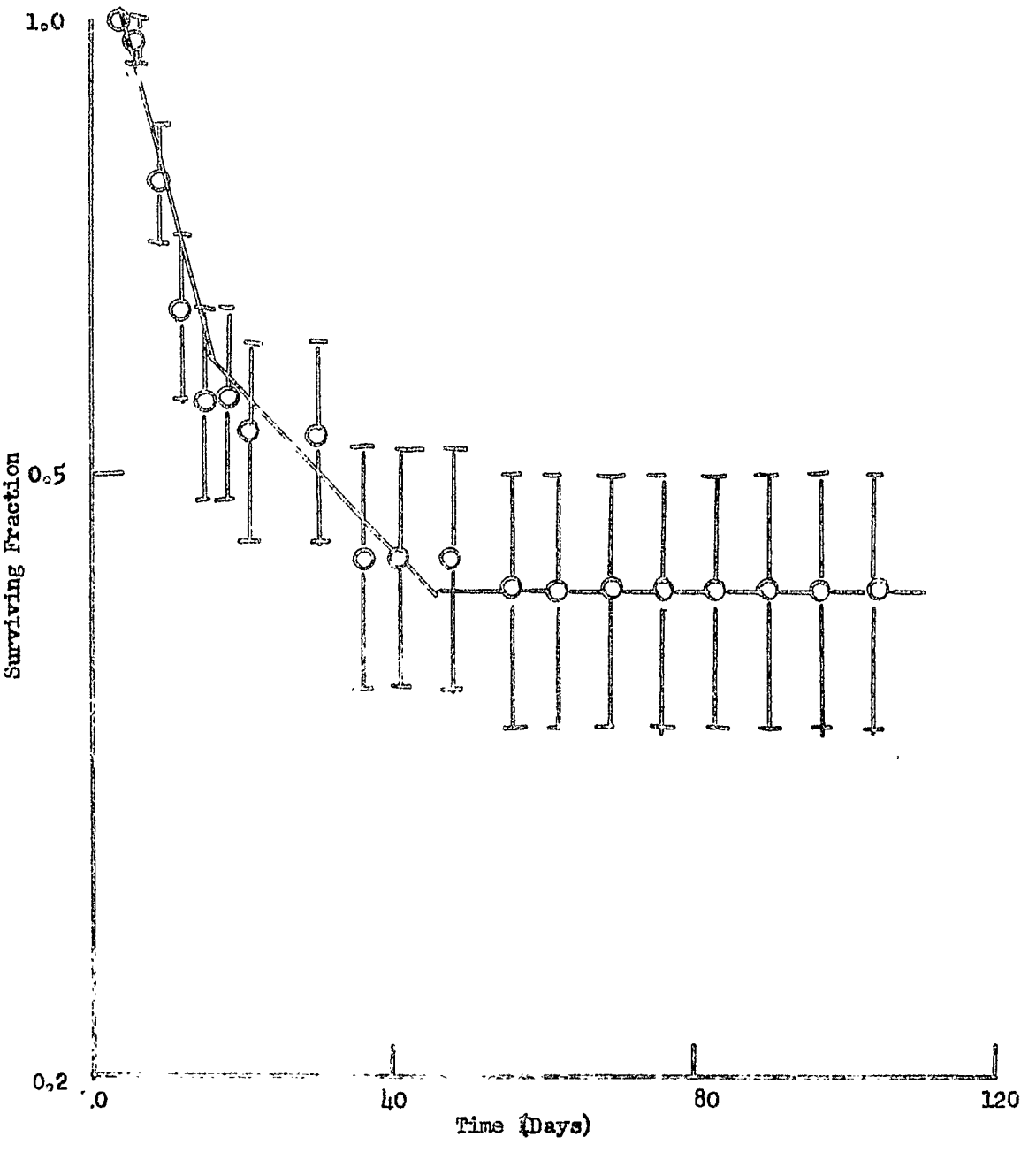

Figure 4. Surviving fraction of mice given vehicle only versus time after 935 rads proton irradiation (Series B).

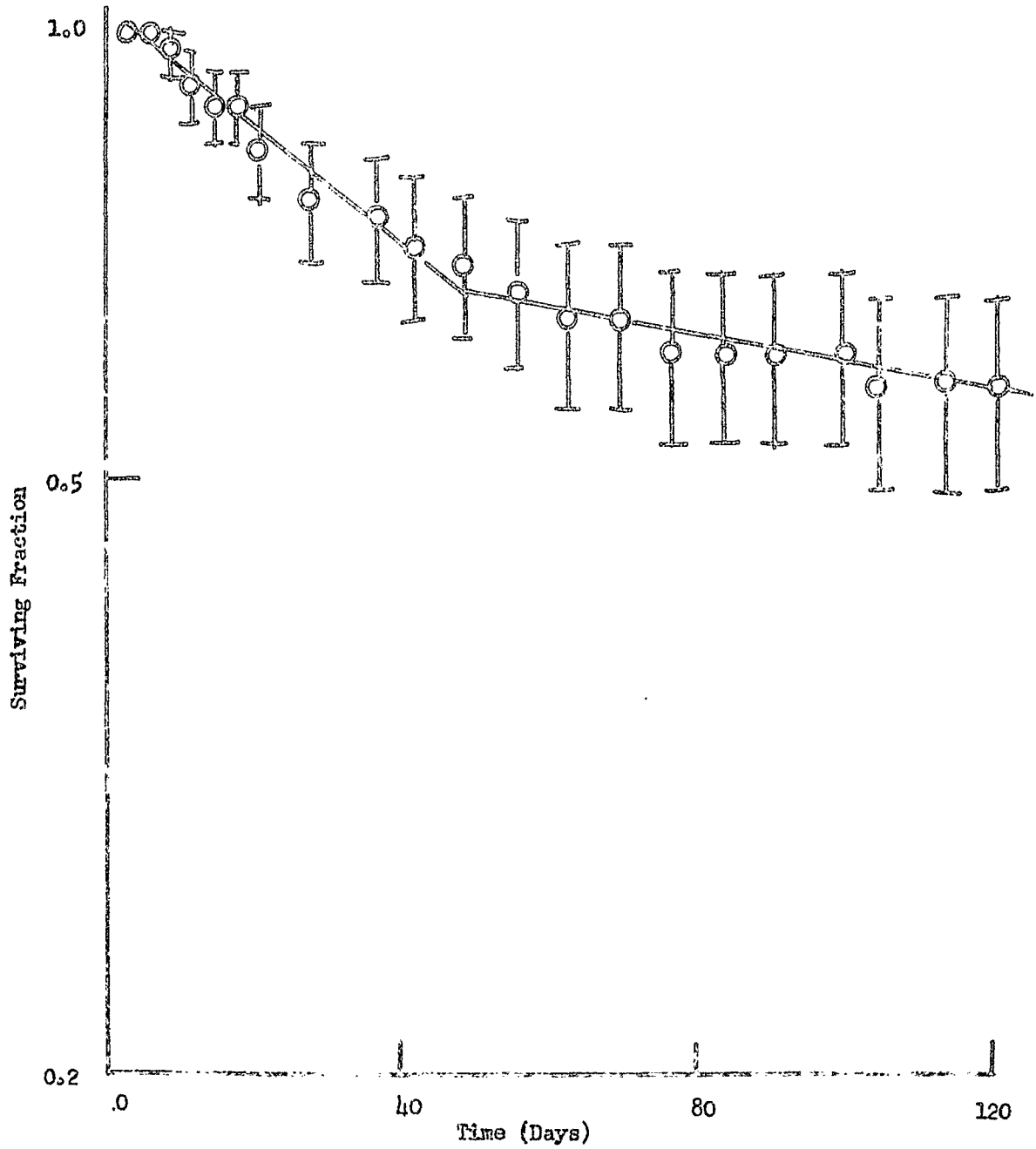

Figure 5. Surviving fractions of mice given vehicle only versus time after 591 rads proton irradiation (Series A).

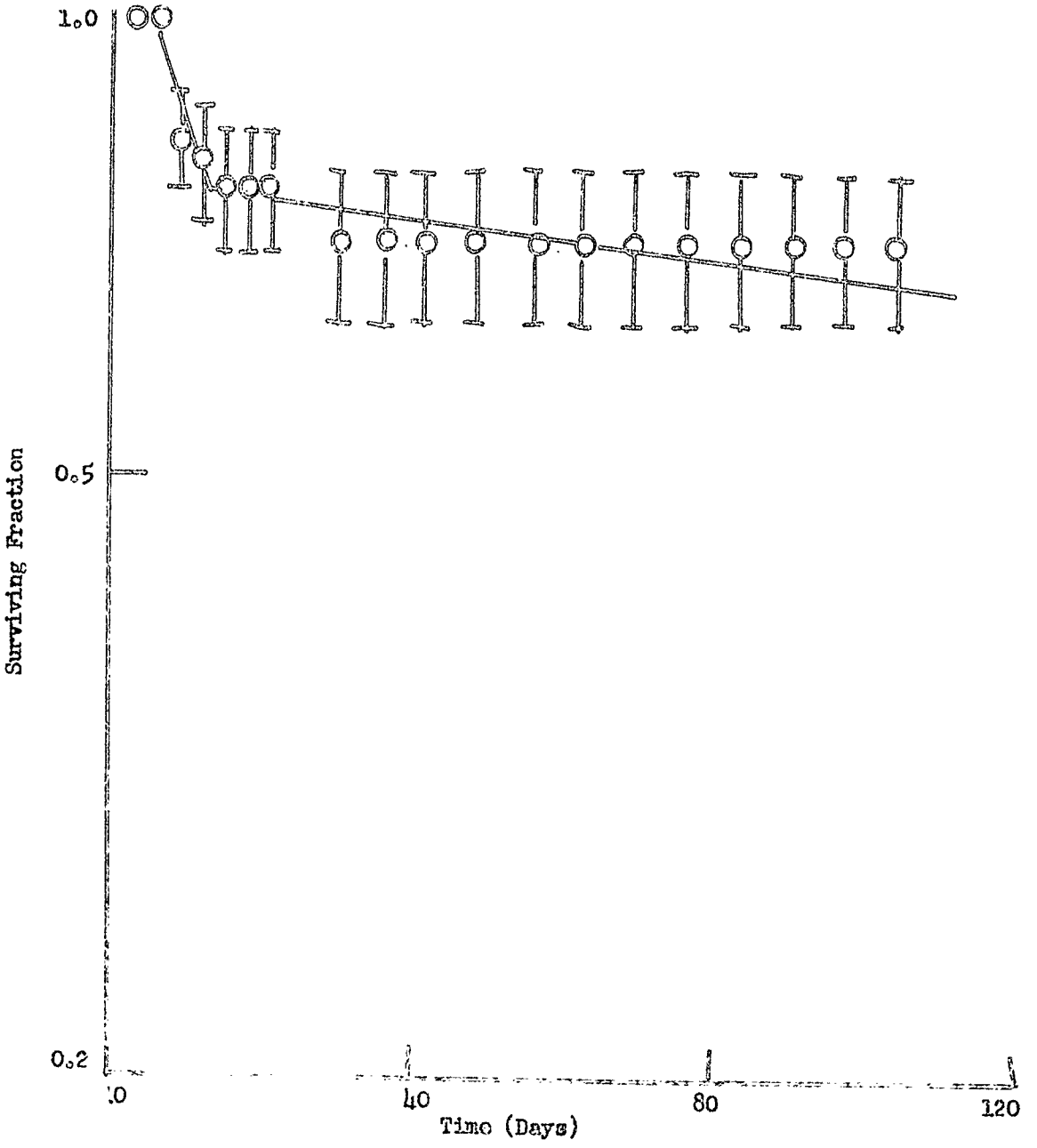

Figure 6. Surviving fraction of mice given vehicle only versus time after 618 rads proton irradiation (Series B).

pre-irradiation treatment with 2-mercaptoethylamine hydrochloride or p-aminopropiophenone is reported.

2. General properties of the survival curves and differences between curves for replicated runs are described.

3. The analytical basis for a detailed comparison of the entire course of survival curves is discussed.

References

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